DISEASES OF POULTRY

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FOREWORD

Owners of profitable poultry flocks owe much of their success to diligence in combating diseases. Poultry are subject to a wide variety of infections, some of which respond readily to treatment, whereas others resist control measures and cause discouraging losses.

A knowledge of the characteristics of each disease is necessary, therefore, as the first step in building up an effective barrier against it. Scientific research already has provided much information of this nature, highly useful as a guide throughout the poultry enterprise. Large expenditures of money, time, and labor are still being made by poorly informed, misguided poultrymen in attempting to control disease by futile means.

With the gradual unfolding of scientific knowledge concerning poultry diseases and parasites, the influence of various infections in connection with breeding, nutrition, housing, and management becomes more and more apparent. This volume contains discussions relating to these and allied phases of poultry raising. The authors, who are specialists in their respective fields, encourage a wider application of sound practices recommended for conquering diseases.

This unusually comprehensive book is intended for students, veterinarians, pathologists, and workers in specialized fields. Trained persons usually are best able to make proper diagnoses of disease conditions and to direct proper courses of treatment.

Finally, the suppression of poultry maladies has a public as well as private aspect. The effect of a loss caused by disease is seen and felt first by the flock owner, but the presence of a transmissible infection is a danger

to neighboring flocks as well. Moreover, large numbers of small individual losses add up to a substantial national total. I trust, therefore, that this book may have a far-reaching influence in improving poultry health, with benefits to the poultry industry and the national welfare.

April, 1943

<div style="text-align:right">

JOHN R. MOHLER

Chief, Bureau of Animal Industry
United States Department of Agriculture
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PREFACE

During the six years that have elapsed since the publication of the fourth edition of Diseases of Poultry in 1959 many new developments have occurred in the system and methods of poultry production. At that time the elimination of many small producers and independent local hatcheries was noted. The trend has continued toward larger production units, and the application of more advanced genetic guidance.

The rapid advances made in the knowledge of poultry diseases by an ever-increasing number of highly trained researchers from various basic disciplines has created a tremendous amount of scientific literature. It is hoped that this volume encompasses much of this information for use by the industry, and that it will serve as a starting point for future research.

We also trust that the book will continue as in the past to be of service to public health workers as it concerns diseases transmissible to man, especially ornithosis, salmonella infections, and Newcastle disease.

The thoughts expressed in the foreword of the first edition, so well written by the late Dr. John R. Mohler in 1943, have not been altered by the passing of time.

Again the editors welcome the new contributors to the fifth edition. To these new authors and to past authors who made possible the undertaking during the last 35 years this book is dedicated.

At this time we express the highest tribute to the memory of Dr. Erwin L. Jungherr, one of the charter authors who was taken from our midst on April 16, 1965, only one month after he had returned the revised galleys of his chapters. Dr. Erwin L. Jungherr's sincerity, research competence, and integrity,

[xi]

and his tireless and patient efforts in cooperating with the editors established a lasting image of respect and friendship for this man.

Again, grateful acknowledgment is made to Director Robert W. Orr of the Iowa State University Library and to Elizabeth A. Windsor and Mrs. Mildred E. McHone of the Reference Department during the extensive bibliographic work in connection with the book.

The counsel and guidance of Mr. Raymond Fassel of the Iowa State University Press and others were most helpful.

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[xiii]

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1

Avian Anatomy

AFFINITIES OF REPTILES, BIRDS, AND MAMMALS

The birds like the mammals arose from the reptiles but from different ancestors. Both classes appeared during the Jurassic, the mammals from the reptilian Subclass Synapsida, Order Therapsida, and Suborder Theriodontia, and the birds from the Subclass Archosauria (a diapsid reptile), Order Thecondontia, and an ancestral line in or close to the Suborder Pseudosuchia. The ancestral birds lived contemporaneously with the dinosaurs and flying reptiles.

Birds and mammals have pursued their evolutionary development independently of each other and by no means can one class be considered either more primitive or more highly developed than the other; each has specialized in different directions. Reptilian cousins of the birds are represented today in *Sphenodon*, and in the crocodiles, lizards, and snakes, whereas all of the synapsid reptilian lines related to

mammals became extinct in the Jurassic. In part mammals seem farther removed from their reptilian progenitors than do the birds because there are no living descenders of the theriodonts. The birds share many structural features with their living reptilian cousins and any real understanding of avian anatomy is dependent upon at least some familiarity with the structure of reptiles.

FIG. 1.1 — A section of skin from the femoral tract of a 20-day-old chicken embryo. a., artery; conn., connective; m., muscle; tiss., tissue. (From USDA.)

INTEGUMENTARY SYSTEM

Histology of the Skin

The skin is composed of two principal layers, *epidermis* and *dermis* (Fig. 1.1), the first derived from the embryonic ectoderm and the second from the mesoderm. The epidermis of birds is thin except in naked or modified areas and is composed of relatively few layers of cells compared to mammals. The dermis also is thin in non-feathered areas protected by overlying plumage; it is considerably thicker in those parts that carry the implantation of feathers. The skin is usually dense and tough in highly vascularized, exposed areas such as ear lobes, comb, and wattles of the chicken and on the nearly naked head and neck of the turkey. Intense keratinization of the epidermis may produce tough, specialized structures such as spurs, claws, scales, and beak.

The epidermis in most parts of the body is composed of but two layers, the *stratum germinativum* and the *stratum corneum*. The basal cells of the germinative layer are larger than those more superficially placed, which are transitional toward the flat-

tened, keratinized, dead cells of the corneal layer. In the epidermis covering the claws, the transition layer may properly be called a *stratum spinosum*; in this location a *stratum granulosum* is also present but in most parts of the body, the transition to the corneum is a gradual one. A *stratum lucidum* and a basement membrane are absent in birds.

The dermis is so variable in thickness and complexity of tissue structure that it does not readily lend itself to a generalized subdivision into layers. In a feathered area (Fig. 1.1) the dermis is slightly more dense and cellular in the area beneath the epithelium than in its deeper parts. Dermal papillae, typical of mammalian skin, are absent in birds except in the feet.

The feather follicles project into the dermis and the smooth muscles that move them are located in this layer. The collagenic connective tissue bundles of the superficial layer intertwine in all directions and usually the layer is well vascularized. Corpuscular types of nerve endings are abundant, particularly Vater-Pacini and Grandry. The dermis in the apteria of the body contains near its lower surface a flat

thin layer of smooth muscle bundles, the ends of which are united by dense bands of elastic tissue, organized in such a way as to form a network structurally capable of moving the nonfeathered skin.

Elastic fibers are scattered among the collagenic fibers and in addition usually form a thin but definite layer near the lower surface of the dermis in a network parallel to the surface of the skin. Elastic tissue forms the tendons that connect the feather muscles to the follicles.

The subcutis may be areolar or adipose tissue. Liebelt and Eastlick (1954) have given names to 16 subcutaneous fat bodies and have illustrated their positions and shapes in the late chick embryo. Much of our basic information on the histology of the avian skin comes from the publications of Moser (1906), Greschik (1916), and Lange (1928, 1931).

Feather Tracts and Spaces

Contour feathers are arranged in rows within tracts (*pterylae*) in the skin of most birds. The tracts are separated by nonfeathered spaces (*apteria*). The arrangement of feathers was first described by Nitzsch (1867) and has been the subject of numerous studies such as those by Pycraft (1898), Steiner (1917), Boulton (1927), Compton (1938), and Humphrey and Clark (1961). The embryonic development of *pterylae* has been investigated by Holmes (1935), Gerber (1939), Wetherbee (1957), and Komarek (1958).

The more important *pterylae* and *apteria* are listed below and those visible in a lateral view of a chicken are shown in Fig. 1.2.

Pterylae

1. Capital tract—head. This tract has numerous subdivisions.
2. Dorsal cervical tract—dorsal surface of the neck.
3. Ventral cervical tract—ventrolateral surface of the neck.
4. Interscapular tract—dorsal mid-region between the shoulders.
5. Dorsal and pelvic tracts—dorsal surface of the trunk.
6. Pectoral tract—ventrolateral surface of the breast.
7. Lateral body tract—side of body near axillary fossa.
8. Sternal tract—ventral side of trunk on each side of the keel.
9. Abdominal tract—ventral side of the abdomen.
10. Humeral tract—dorsal surface of upper arm near shoulder.
11. Alar tract—dorsal and ventral surfaces of the wing. This tract has numerous subdivisions.
12. Femoral tract—thigh region.
13. Crural tract—leg region.

Apteria

1. Lateral cervical apterium—side of the neck, between dorsal and ventral cervical tracts.
2. Ventral cervical apterium—ventral surface of neck, between right and left ventral cervical tracts.
3. Scapular apterium—between interscapular and humeral tracts.
4. Lateral pelvic apterium—between pelvic and femoral tracts.
5. Lateral body apterium—space surrounding the lateral body tract.
6. Pectoral apterium—between pectoral and sternal tracts.
7. Sternal apterium—over the keel, between right and left sternal tracts.
8. Crural apterium—knee region, between crural and femoral tracts.

Feather Types

Feathers vary in complexity from a simple down feather that is entirely plumulaceous (fluffy) to the intricate contour feather that is partly pennaceous (closely knit texture) (Fig. 1.3). A semiplume is an intermediate type of feather, characterized by plumulaceous structure but having a distinct rhachis. The rhachis is that part of the shaft that bears filaments (barbs). In the contour feather the barbs project from the two sides at about a 45° angle and in turn lesser filaments, the barbules, project distally and proximally from the margins of each barb at about a 45° angle. The result is that the barbules cross each other at about a 90° angle: one set of barbules carries hooklets that loosely engage the flanges of the barbules that cross beneath them. It is this mechanism that gives stiffness and cohesiveness to the vanes of contour feathers. At the base of the feather, the hooklets and often the barbules themselves are absent and because the barbs are unrestrained they produce a fluff or plumulaceous portion of a feather.

At the upper end of the calamus (im-

Comb
Spike
Blade
Body
Base
External nasal opening
Rictus
Internasal tr
Wattle

Adult remiges
XI
Primary remiges, 1–X
Under primary coverts
Axial (or key) feather
Secondary remiges, 1–17 or 18
Under secondary coverts
Under marginal coverts of the propatagium

Caput tra
Temporal apt.
Ear care
Ear opening
Ear lobe

Dorsal cervical tr
Lateral cervical tr
Interscapular tr
Ventral cervical apt.
Ventral cervical tr
Lateral body tr
Pectoral tr
Lateral body tr
Pectoral apt.
Sternal tr
Knee apt.
Sternal apt.

Internal crural tr

Alar apt
Subhumeral tr.
Metapatagium
Dorsal tr.
Pelvic tr
Hip reg
Lateral pelvic apt.
Femoral tr.
Postpelvic tr
Upper caudal apt.
Tail coverts
Rectrices
Under caudal apt.
Abdominal apt.
Abdominal tr.
Crural apt.

External crural tr

RAYMOND LURIA

Ankle
Metatarsus
Metatarsal spur

Toes
Metatarsal pad
I
II
IV
III

FIG. 1.2 — Feathered tracts (pterylae) and nonfeathered areas (apteria) shown on a lateral view of the chicken. Dash lines are placed to show arbitrary boundaries in long tracts extending over two or more parts of the body. apt., apterium; Ext., External; reg., region; tr —trs, tract—tracts. (From USDA.)

FIG. 1.3 — Parts of a typical contour feather—chicken. (From USDA.)

planted end of the shaft) a miniature feather (afterfeather, s. aftershaft) emerges from the inferior surface and parallels the rhachis. This structure is present in chickens and turkeys, rudimentary in ducks, and absent in pigeons (Miller, 1924). Afterfeathers are absent from natal down of all species.

The largest contour feathers are the flight quills on the wings and tail: the remiges and rectrices respectively. There are 10 primary remiges implanted on bones of the hand of most birds. On the wrist there is a vestigial feather, the carpal remex, that is frequently overlooked. The ulna of the forearm carries 16–19 secondary remiges in chickens, the first of which is shorter than the feathers adjacent to it and is often called the axial or key feather. There are 6–8 pairs of rectrices in the chicken; the median pair of which, in the

male, forms the greater sickles. Smaller feathers, the coverts, overlie the bases of remiges and rectrices.

Contour feathers are modified in various ways; the outer margin of the hackle feathers of the cock is devoid of barbules. The barbs of auricular feathers are stiff and far apart which provide an effective screen but do not interfere with the passage of sound. Bristle feathers consist of a shaft that is either entirely bare or has a few barbs at the base. They are found on the carunculate skin of the head and neck of turkeys and at the corners of the mouth in various other birds. Bristles on the eyelids are known as eyelashes or, if tiny, as cilia.

Down feathers form the first plumage of birds; they are also present later at the margins of the tracts in chickens and pigeons, and among the contour feathers
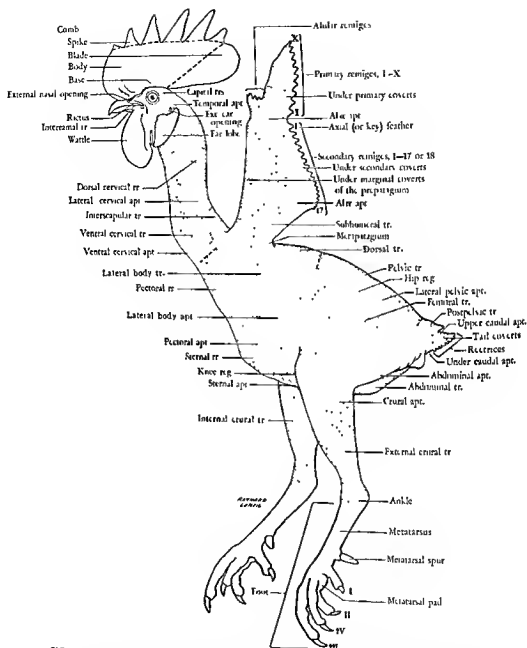
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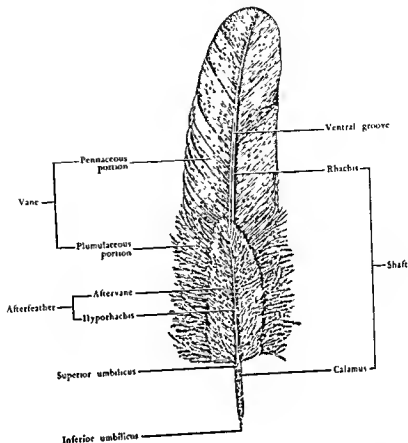
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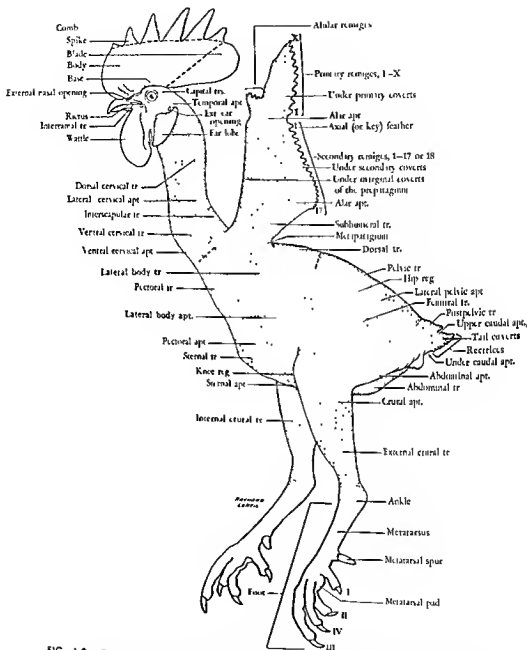
in ducks. Their long, flexible barbs usually arise from a very short rhachis. In the natal downs of certain birds, the barbs arise from the upper end of the calamus. The barbs bear barbules composed of a series of simple segments without hooklets.

After a chicken has been plucked there remain numerous hairlike feathers called filoplumes. Typically they have a calamus and a slender, long rhachis with a tuft of barbs at the tip. They bear no afterfeathers in any species of bird. Their function is unknown. In the chicken they are longest on the neck and back and are most numerous around the remiges.

Feather Musculature

The contour feathers of the body can be readily elevated or depressed, as seen in the hackles of the crowing cock and also seen in the body feathers when bathing or dusting. These actions are controlled by a network of smooth muscles innervated through the sympathetic nervous system (Langley, 1904; Ostmann et al., 1963). To the unaided eye the bundles of smooth muscles join all adjacent follicles and in the network have the arrangement of parallelograms. In some fiber-tracts, muscle bundles cross diagonally joining opposite corners.

A dissection of the feather musculature under the microscope makes it evident that the bundles of muscles are arranged as antagonists, erectors, and depressors (Stettenheim et al., 1963). One end of a muscle unit attaches to the bottom of one follicle and the other end to the top of an adjacent follicle. Another unit between the same two feathers will have the reverse arrangement (Fig. 1.1). It is evident, therefore, that the contraction of a set of muscles will erect two adjacent feathers and an opposite crossing set will depress the same two feathers. Smooth muscles do not encircle a follicle; there are bundles of elastic tissue that link the smooth muscles to the outer surface of the follicle that contains

within its wall elastic and collagenic connective tissues (Fig. 1.1).

Integumentary Structures

Comb, wattles, ear lobes, beak, scales, claws, spurs, and pipping tooth of chickens, leader (s. snood), carunculate skin and beard of the turkey, and the beak, nail and lamellae (s. strainers) of the duck are specialized integumentary structures. They show the basic plan of the integument found in feathered parts of the body. The leader of the turkey has an erectile-type vascular tissue composed of helicine arteries and sinusoidal capillaries near the surface of the organ. Bundles of smooth muscles extend lengthwise through the leader.

The comb of a chicken has neither helicine arteries nor longitudinal muscles. Between the central connective tissue axis of the comb and the peripheral capillary layer is a broad band of muco-fibrous tissue in the adult male and the laying female chicken. According to Champy and Kritch (1925 and 1926) this mucoid tissue disappears in the capon and in the female out of production. They suggest (1926) that turgor is brought about in the comb of the cock by the production of an edema in the muco-fibrous layer, that this compresses the small, thin-walled veins producing a blood stasis in the sinusoidal capillary layer.

The wattle, like the comb, has a central axis of dense connective tissues bearing many large vessels and nerves. The deep dermal layer is composed of very loose connective tissue and the superficial layer is well vascularized with small vessels. A muco-fibrous layer may be present under the same conditions that produce its presence in the comb. The deep dermal layer of the ear lobe is dense collagenic tissue with fiber bundles running in three directions. The superficial layer is highly vascular even in the white ear lobe of the leghorn. Champy and Demay (1930) suggest that the white coloration of the ear lobe

and some of the caruncles of the turkey are due to a sclerotic condensation of connective tissue.

Scales of birds, like those of reptiles, develop as thickenings of the *stratum corneum*. Between scales the corneal layer is depressed and usually thinner than at the surface of the scale and is more flexible. Large scales may overlap slightly but generally they lie in the same plane as the epidermis. There is continuity of the corneal layer of the scales with that of claws and spurs and the tissue components of the beaks are the same as a large thickened scale.

Only holocrine glands occur in avian skin, the uropygial on the dorsal surface of the tail and the "ear fold" within the external canal (Plate, 1924). The uropygial gland produces a yellow ceruminous secretion that is emptied into a large central cavity and is conveyed to the outside through ducts carried by the uropygial papilla. The secretory units are straight tubules with their closed ends against the connective tissue wall of the gland. Generative cells form a single layer at the periphery of each tubule and as the cells move centrally, lipid spheres form within the cytoplasm. These increase in number and in the cells that contain them enlarge. At the lumen of the tubule the cells disintegrate, releasing their contents.

SKELETAL SYSTEM

Differences in Skeleton Between Birds and Mammals

There are many differences in the skeletal system of birds and that of mammals. For example, most mammals have 7 cervical vertebrae, whether the neck is short as in a whale or long as in a giraffe, but in birds the number varies from 8 to 25 and usually has a relationship to the length of the neck. The total number of vertebrae in birds varies from about 40 to over 60 (Bellairs and Jenkin, 1960; Romanoff, 1960). In mammals only thoracic vertebrae bear movable ribs, but in birds cervical and

lumbar vertebrae may carry movable ribs as well as those of the thorax. The pubic bones of birds are directed posteriorly instead of anteriorly and are spread far apart, so that a *symphysis pubica* is absent. Extensive ankylosis of dorsal vertebrae is common in birds but in mammals is found only in the sacral region. The differences between the head skeleton of birds and mammals are indeed numerous, and are related to their reptilian progenitors. (For a good general reivew of the avian skeletal system, see Reynolds, 1913.)

Axial Skeleton

The neck and tail are flexible but the body (thorax, lumbar, and sacral regions) has only one movable vertebra throughout its length in the chicken. The relatively rigid body gives a firm fuselage for the wings and landing gear.

There are 16 or 17 cervical vertebrae, beginning with the atlas (Fig. 1.4). The last of these in older speeimens may be partially or entirely fused with ankylosed thoracic vertebrae. The centrum bears a heterocoelous articulation, namely saddle-shaped, in which the anterior face is concave horizontally and convex vertically but this changes to a nearly acoelous condition in the coccygeal vertebrae.

On the dorsal side of each cervical vertebra there is a pair of prezygapophyses with articular surfaces upward and a pair of postzygapophyses with articular surfaces downward. Through the series of cervical vertebrae the right and left components of the neural spine may be fused, separated, or absent. The right and left hypapophyses (ventral processes from the centrum) may be fused in the mid-line or remain apart. The hypapophysis of the last cervical is often joined with that of the succeeding fused thoracic vertebrae. On the lateral surface, close to the fore margin of each cervical vertebra except on the atlas and axis, are two transverse processes encircling the vertebral artery, forming the transverse foramen. The processes

Phalanx 2, digit III
Phalanx 1, digit III
Phalanx 1, digit IV
Phalanx 2, digit II
Phalanx 2, digit II
Metacarpus III
Metacarpus IV
Process of metacarpus II
Os ulnare
Os radiale
Ulna
Radius
Olecranon
Entepicondyle
Ectepicondyle
Humerus
Scapula
Uncinate process
Vertebral member of rib
Median dorsal ridge
Trochanter of femur
Iliolateral process
Ilio-Ischiatic fenestra
Pygostyle
Iliocaudal spine
Obturine foramen
Ischium
Ischiatic process
Pubis
Femur
Caudal end of sternum
Fibula
Tibiotarsus
Lateral condyle (malleolus) of tibia
Ossified tibial cartilage
Lateral calcaneal ridge of hypotarsus
Os tarsometatarsus, II IV
Terminal phalanx, digit I
Basal phalanx, digit I
Basal phalanx, digit IV
Terminal phalanx, digit IV

Supra-occipital eminence
Sclerotic ring
Jugal bar
Hyoid
Styliform process
Deltoid crest
Bicipital crest
Tricosteal canal
Vertebral member of rib
Os coracoideus
Clavicle (furcula)
Anterolateral process of sternum
Sternocoracoid joint
Hypocleidum
Sternal member of rib
Posterolateral
process of sternum
pars lateralis
pars medialis
Lateral sternal notch
Medial sternal notch
Carinal spine
Patella
Keel (carina)
External cnemial crest
Metatarsal spur
Accessory metatarsus for metatarsus, II
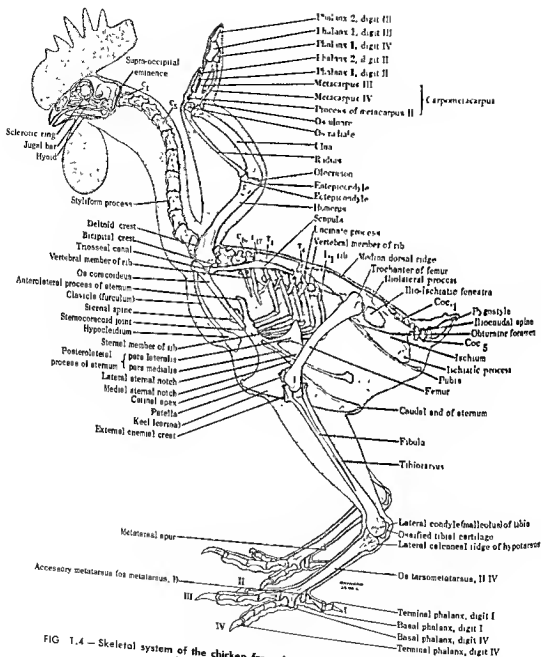II
III
IV

FIG 1.4 – Skeletal system of the chicken from the left side. C., cervical; Coc., coccygeal;
L, lumbar; T, thoracic. (From USDA.)

are derived from a dorsal component, the diapophysis, and a ventral component, the pleurapophysis. The latter, on some vertebrae, carries a styloid process projecting posteriorly. The diapophysis carries an articular surface for the tubercle of the movable rib and the pleurapophysis forms the vertebral member (dorsal part) of the rib, including the head. In a skeleton of a chicken 6 to 8 weeks of age, a plate at the side of the centrum, called the parapophysis, supports the pleurapophysis and in older birds fuses with it, or becomes the facet area for the head of the movable rib.

Mivart (1895), Howard (1929), and Zusi (1962) are some who have presented terminology for the adult condition of the vertebrae of birds but this is best interpreted through studies of embryology (Piiper, 1928; Williams, 1942; Romanoff, 1960) and comparative anatomy (Goodrich, 1958).

The vertebrae and their lateral processes, the ribs and sternum, form a skeletal enclosure for thoracic viscera. The last two cervical vertebrae bear movable vertebral ribs. Arbitrarily the true thoracic ribs generally have been considered as those that have both vertebral and sternal members and together they form a complete rib that extends from a vertebra to the sternum. (In mammals the costal cartilage represents the sternal member.) Uncinate processes, directed posteriorly, are carried by the vertebral member of the last cervical and all of the thoracic ribs but not by the lumbar rib at the end of the series. These processes provide origin for serratus muscles to the scapula and attachments for intercostal muscles. The lumbar ribs (posterior floating ribs) are either incomplete or articulated with a caudal thoracic rib (rather than with the sternum). The first pair of lumbar ribs is complete and the second may be nearly complete or a small rudiment.

On the basis of its ribs, the chicken has 4 (sometimes 5) thoracic vertebrae (Fig. 1.4). The last cervical and the first 3 thoracic are ankylosed in older birds. The hypapophysis lies between the right and left lungs and forms an origin for ventral neck muscles. The last thoracic vertebra is movable.

The 4 lumbar, 5 sacral, and the first 6 caudal vertebrae are fused into an immobile dorsal plate, the synsacrum. The separate vertebrae are clearly shown in a two-month-old chicken. The fused plate, along with the pelvic arch, composed of ilium, ischium, and pubis, forms the pelvis. The lumbar vertebrae have robust dorsal and ventral processes (diapophyses and pleurapophyses respectively); the last in the series is especially heavy. The first in the series bears a pair of lumbar ribs. The sacral vertebrae have dorsal but lack ventral processes and may be so completely fused in older birds that they are difficult to count but may be identified by the foramina through which the sacral nerves emerge. Caudal vertebrae form the tail; the 6 incorporated within the synsacrum have both dorsal and ventral lateral processes. Posterior to these, there are 6 coccygeal (posterior caudal) vertebrae and a terminal flattened bone, the pygostyle, representing a fusion of about 6 additional caudal vertebrae. The articular surfaces of the centra of the free coccygeal vertebrae allow limited movement. These vertebrae, except the last, have flat transverse processes. Sometimes these processes of the first coccygeal vertebra articulate with the ilia. A chicken, therefore, has a total of approximately 42 vertebrae, exclusive of the pygostyle.

Pectoral Girdle and Wing

The pectoral arch, composed on each side of scapula, coracoid, and clavicle is a tripod-shaped structure with the shoulder joint at its apex (Fig. 1.4). The right and left clavicles are fused ventrally by the hypocleidium and united to the sternal spine by a ligament. Lateral to the spine is a deep transverse groove into which the broad base of the coracoid is implanted and which allows limited antero-posterior movements. The dorsal end of the clavicle articulates on its lateral face with the head

Phalanx 2, digit III
Phalanx 1, digit III
Phalanx 1, digit IV
Phalanx 2, digit II
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Metacarpus IV   } Carpometacarpus
Process of metacarpus II
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Humerus
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Neural process
Vertebral member of rib
Median dorsal ridge
Trochanter of femur
Iliolateral process
Ilio-ischiatic fenestra
Coc₁
Pygostyle
Iliocaudal spine
Coc₅
Obturator foramen
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Ischium
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Posterolateral   } pars lateralis
process of sternum   pars medialis
Lateral sternal notch
Lateral carinal notch
Patella
Keel (carina)
External cnemial crest

Metatarsal spur

Accessory metatarsus for metatarsus II

III
IV

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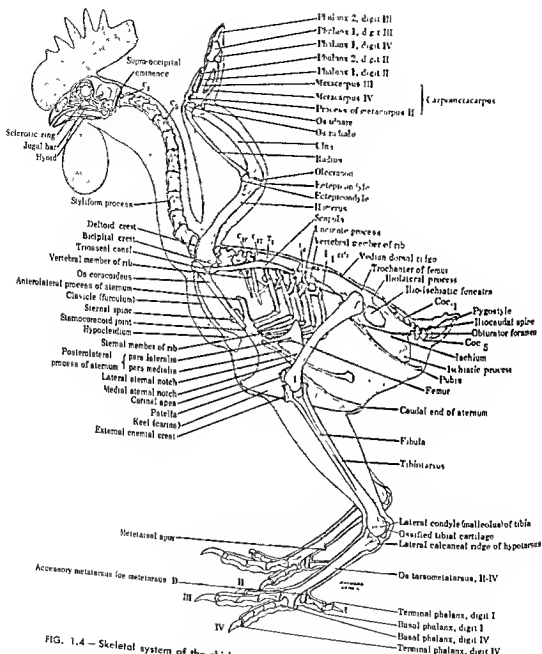
of the coracoid. A space formed by the heads of the 3 bones of the pectoral girdle is the triosseal canal, through which passes the tendon of the supracoracoid muscle, the chief action of which is to elevate the wings. A large cartilaginous pad forms the glenoid fossa supported on the articular surfaces of the coracoid and scapula.

The sternum is composed of a narrow, median plate with antero- and posterolateral processes and a deep, triangular keel (carina) (Fig. 1.4). The thoracic ribs are implanted on the costal margin of the sternum between the basal ends of these processes. The posterior process of the chicken has two horns, median and lateral, and these form boundaries for the median and lateral notches. The sternal plate has a sternal spine and the keel has a carinal apex at the anterior extremities; between the two is a C-shaped margin designated by Howard (1929) as the anterior carinal margin The sternum shape is extremely variable among birds (Knopfli, 1918)

The bones of the wing are homologous with those of the arm and hand of other tetrapods, and the humerus, radius, and ulna are readily recognizable. The anterior margin of the proximal end of the humerus has a triangular flange, the large deltoid crest and posterior margin has a tuberosity, the bicipital crest (Fig. 1.4) that carries a large pneumatic foramen on its medial surface. The humeral diverticulum of the interclavicular air sac enters the humerus through this foramen (Fig. 1.8). At the distal end of the humerus are the radial and ulnar condyles, separated by an intercondylar furrow. Ect- and entepicondyles and their prominences lie dorsal and ventral to the trochlea along the edge of the bone. Several extensor and flexor muscles of the forearm have their origin from these tuberosities. The wrist bones of the hand have been reduced to a radiale (s. os scapholunatum) and an ulnare (s. os cuneiforme) in the adult but additional wrist bones are present in the embryo. During growth and development

some of the distal carpals fuse with the 3 metacarpals to form the carpometacarpus, the largest bone of the hand. (See Romanoff, 1960, for review of development.) In young chickens the unfused carpal bones may be mistaken for the epiphyses of the metacarpals.

Embryologic studies have provided evidence that the avian hand has retained digits 2, 3, and 4, having lost the first and last digits. The short digit on the anterior edge of the wing supporting the 3 to 4 alular remiges is frequently labeled "thumb (s. pollex)" whereas actually it is the second digit (index finger). Departure from the earlier concept of digits 1, 2, and 3 was brought about by the convincing embryologic studies of Montagna (1945) and Holmgren (1955) and a review by Romanoff (1960).

The bony knob at the base of the index finger is metacarpal 2 on which is inserted the tendons of propatagial muscles, m. extensor metacarpi radialis and m. extensor indicis longus (s. m. extensor pollicis longus) (Hudson and Lanzillotti, 1955). The metacarpal bones are fused with a long space between metacarpals 3 and 4. Metacarpals 2 and 3 bear 2 phalanges, and metacapal 4 bears 1 in the chicken. The distal phalanx of digit 2 bears a short claw in chickens but not in turkeys (Fisher, 1940).

Pelvic Girdle and Extremity

The pelvis is a compound structure composed of the synsacrum and bones of the pelvic girdle. (See Lebedinsky, 1913, and Howard, 1929 for names of parts.) The acetabulum and its adjacent processes mark the division between anterior and posterior parts of the pelvis. The preacetabular part of the ilium is a thin, spoon-shaped plate, the medial margin of which is the crest that extends caudolaterally to the iliolateral process above the acetabulum. The postacetabular part lies in two planes. The medial portion joins the

synsacrum in forming a horizontal plate and the lateral portion with the ischium forms the side wall of the pelvic girdle. The fusion of these two bones is interrupted by a large, elongate ilioischiatic fenestra. Immediately above the acetabulum is the antitrochanter derived from both ilium and ischium. Below and forward the well-developed pectineal process of the ilium is present, to which is attached the ambiens muscle. The ventrocaudal extension of the ischium has been named the ischiatic process (s. ischial angle).

The pubis is a long, slender bone that arises from the ventral part of the acetabulum, and extends beyond the ischium where it curves toward the median plane. Anteriorly the pubis is separated from the ischium by the obturator foramen and posteriorly by the ischiopubic fenestra. The interpubic space varies with the sex of the bird and is widest in laying females. On the ventral side of the pelvic arch are several cavities; lateral to the lumbar vertebrae are the lumbar foveae; caudal to this is an extensive iliac fossa (s. renal depression), separated into anterior and posterior parts at the level of the lateral process of the first caudal vertebra. The fossa continues to the iliocaudal spine but its caudal part is separated from the viscera by the planum anale which has as its cephalic boundary the conspicuous posterior ischiosacral crest.

The thighs of birds are closely pressed against the side of the trunk and in grebes, penguins, and loons only the part of the leg beginning at the knee is distinctly separated externally. The femur of the fowl is sufficiently similar to that of mammals so that its principal parts are readily recognizable, although homologies of the trochanters are controversial (Howell, 1941).

The distal end of the femur articulates medially with the head of the tibiotarsus, and laterally with the head of the fibula. The fibula ends about one-fourth of the distance short of the tarsal (hock) joint.

The knee includes a patella as in mammals. Anterior to the head of the tibiotarsus are sharp ridges, the external (outer) and internal (inner) cnemial crests, to which are attached both thigh and leg muscles. Parker (1884–94) considers these crests to epiphyses.

There are no free tarsal bones in the adult fowl; during development and growth these have fused with the tibia proximally and the metatarsus distally (Nielsen, 1963). Therefore, the intertarsal (hock or ankle) joint involves only tarsal bones rather than an articulation between the long bone of the leg and the ankle bones. During the first few months after hatching the proximal tarsal bone remains as a separable cap on the end of the tibia and as in the arm, superficially resembles an epiphysis. The possible conversion of a sesamoid into a traction epiphysis has been described by Barnett and Lewis (1958).

The tibial cartilage on the distal posterior surface of the tibiotarsus forms a restraining groove for the tendons that extend the foot and flex the toes. The medial part of this cartilage is ossified in older chickens. On the proximal posterior surface of the tarsometatarsus is located the hypotarsus, a separate bone in the young bird that later unites with the tarsometatarsus. It also by its tendinal canal and its ecto- and endocalcaneal ridges separates and guides the tendons for numerous flexor muscles of the toes.

The fifth toe is absent in all birds, but most species have retained 1 to 4 (one or more supernumerary toes are present in several breeds of chicken). A short first metatarsus (accessory metatarsus) is located at the base of the hallux. The distal end of the tarsometatarsus is divided into its original 3 component bones, each articulating with a basal phalanx by a ginglymus (hinge) joint. In many birds each toe has one more phalanx than its digit number; the hallux has 2; the second toe, 3; the third toe, 4; and the fourth toe, 5. Most
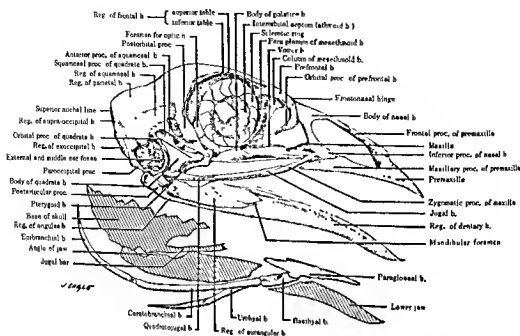
FIG. 1.5 — Bones of the skull, face, jaws, and hyoid of an adult male turkey. b., bone; n., nerve; proc., process; Reg., Region. (From USDA.)

of the terminal phalanx forms a bony core for the claw.

Skeleton of the Head

The head includes cranium, face, jaws, and hyoid. The bones of the head reflect particularly well the reptilian ancestry of birds. The cranial bones in the adult are completely fused and most of the suture lines disappear (Fig. 1.5), but the separate bones of the head can clearly be distinguished in the young specimen, the development of which has been described by Jollie (1957).

The premaxilla has 3 processes, maxillary, palatine, and frontal, all directed caudally. The nasal bone lies laterally on the frontal process (s. nasal process) of the premaxilla and has two processes of its own, superior and inferior. The maxilla in birds is relatively small; there are 2 processes, a zygomatic to the jugal bar (upper jaw) and a palatine.

The prefrontal bone and its orbital process have been designated the lacrimal

bone by many, but Gregory (1920) has presented evidence that mammals retained the reptilian lacrimal, losing the prefrontal, and that the reverse took place in the evolution of birds. The orbital process lies between the nasal and orbital cavities and supports the attachments for the oblique eye muscles. The prefrontal bone and its process are highly variable in size and shape among various species of birds.

The paired frontal bones are large; their anterior ends as well as the caudal tips of the premaxillae rest upon the upper end of the mesethmoid. The fused parietals located at the caudal part of the cranium are flanked by a pair of squamosal bones and caudally by the base of the head, a median supraoccipital and a pair of exoccipital bones. These, with the basioccipital bound the foramen magnum. The occipital condyle is a single median structure, in contrast to the paired condition in mammals.

The interorbital septum (s. ethmoid bone) is the thin partition that separates

right and left orbital cavities. The palpable posterior boundary of the orbit is a pointed postorbital process of the alisphenoid frequently fused with the anterior process of the squamosal bone. The ear has a complicated osteology; its walls and parts are derived from the epiotic and episthotic bones on the inner surface of the supraoccipital, and the prootic beneath the squamosal. The columella and semicircular canals are discussed later.

The quadrate bone, said to be homologous with the incus of mammals, has two processes and is the point around which most actions of the jaw pivot. The squamosal process articulates with the prootic bone. The orbital process of the quadrate articulates with the pterygoid and this in turn with the palatine. The body of the quadrate articulates with the quadratojugal and suspends the lower jaw. The upper jaw is elevated at the frontonasal hinge by means of a rotating movement of the quadrate, which is transmitted through the jugal bars and the palatines (Zusi, 1962). Movements of the quadrate also act on the lower jaw.

The embryonic components of the lower jaw—articular, angular, surangular, prearticular, splenial, and dentary—are fused early in life, but some of these are still distinguishable in the adult. It is characteristic of birds that the lower jaw, approximately in the center of its lateral surface, is perforated by a mandibular foramen.

The hyoid is composed of a group of bones arranged as a median axis and a pair of long, slender horns that curve upward around the base of the head. In the mid-line the basihyal articulates anteriorly with the paraglossal, a structure unique in birds (Bellairs and Jenkin, 1960) and posteriorly with the urohyal. The horns, composed of a basal ceratobranchial and a distal epibranchial, articulate on the sides of the basihyal. The hyoid supports the tongue and its musculature.

A ring of 12–16 (median 14) overlapping sclerotic plates embedded in the eyeball encircles the iris (Lemmrich, 1931;

Curtis and Miller, 1938; Nelson, 1942; Jollie, 1957). The variability of number and overlap has been studied by Coulombre et al. (1962). The sclerotic ring in birds is inherited from reptiles. In the chicken and many birds it is composed of small plates but in hawks, owls, and other raptorial species the plates are large and arranged to form a tube.

MUSCULAR SYSTEM

The following enumeration based on the muscles of the whooping crane (Fisher and Goodman, 1955) lists the skeletal muscles in various parts of the body and approximates the number of these muscles present in the chicken:

Head, jaw, and adjacent neck	20
Tongue, hyoid, and trachea	18
Eye globe, lids, and a dermal muscle to the ear	12
Wing and pectoral girdle	48
Leg and pelvic girdle	42
Abdominal and thoracic wall	6
Vertebral column including remaining neck	15
Tail	10
Total	171

Numerous individuals have contributed to our knowledge of avian musculature (Helm, 1881; Shufeldt, 1890; Fürbringer, 1888; Gadow and Selenka, 1891; Mudge, 1902; Boas, 1929; Hudson, 1937; Hudson et al., 1955, 1959, 1964; Fisher, 1916; Berger, 1952; Davids, 1952; Den Boer, 1953; Rooth, 1953; Starck and Barnikol, 1954; Burggraaf, 1954; Burggraaf and Fuchs, 1954, 1955; Fuchs, 1954, 1955; Goodman and Fisher, 1962; Zusi, 1962). Many of these papers include synonymy. The developmental sequence for the muscles of thigh, leg, and foot of the chicken has been studied by Romer (1927) and Wortham (1918), and the development of muscle groups of the wing by Sullivan (1962).

Some muscles of birds have no equivalent in mammals, largely because of differences in skeletal structure and in func-

tions of body parts, for example: the ilium of mammals does not extend in either direction much beyond the acetabular region, whereas in birds the ilium is extremely long. Hence the iliotrochanteric muscles are peculiar to birds and not represented in mammals or at least cannot be definitely homologized (Hudson, 1937). The ambiens muscle, which originates on the pectineal process, is peculiar to birds and reptiles and is absent in mammals. A broad, flat, triangular muscle, the iliotibialis, covers much of the lateral surface of the thigh. It is divisible into anterior, medial, and posterior parts. Gadow (Hudson, 1937) considers the anterior part as absent in mammals, the middle part as homologous with the *m. tensor fasciae latae* of mammals and the posterior part as homologous with the *m. gluteus maximus*. In birds the gastrocnemius has three parts. The external and medial parts may represent the *m. gastrocnemius* of man and the internal part may be homologous with the *m. soleus* of man.

In Fig. 1.6 is shown the superficial muscles visible from the side. The terminology for wing, limb, and girdles follows Hudson (1937) and Hudson *et al.* (1955, 1959, 1964), and that for head, hyoid, neck, and tail has been taken from various authors but mostly from Fisher and Goodman (1955). The names of muscles of breast, shoulder, pelvis, and thigh are presented in Tables 1 and 2 and include a brief statement of origin and insertion of each muscle.

Medial to the eye globe are two muscles in addition to the six found in mammals, i.e., *m. quadratus nictitantis* and *m. pyramidalis nictitantis* that are involved in movement of the nictitating membrane (Fig. 1.7).

The bellies of the muscles of the appendages are concentrated near their respective origins, thus shifting their weight closer to the center of gravity. The reduction of digits in the wing is a second factor in the fusion of muscle masses and their

consequent multitude of origins. Centripetal placement of the muscle masses tends to produce long tendons, and in the legs especially these are guided by well-developed fibrous sheaths, loops, grooves, and canals in bone and cartilage. Sesamoid bones are common and many tendons become ossified.

Breast muscle is composed of fibers of two size groups: the large are light in color, the small are dark. In addition there are many cytochemical differences (Watzka, 1939; George and Naik, 1957, 1958, and 1959; George and Talesara, 1962).

VASCULAR SYSTEM

Heart

The avian heart has its axis in the median plane of the body. Its ventricles are nearly surrounded by the lobes of the liver. Like the mammalian heart, it is four-chambered and the circulation of blood through these chambers is similar in the two classes of vertebrates. Some of the distinctive features of the heart in birds, as found by Kern (1926), Petrén (1926), Uchiyama (1928), and Westpfahl (1961), are as follows: (1) Relative to body weight, it is heavier in birds than in mammals or reptiles, (2) neither a *ductus arteriosus* nor a *ligamentum arteriosum* is present in chickens but both structures have been reported for other species of birds, (3) a distinguishable *fossa ovalis* fails to persist after the closure of the *foramen ovale* in the embryo, (4) plaques of cartilage are normally present in the aorta at the level of the semilunar valves and sometimes in the wall of the adjacent common pulmonary artery, (5) most commonly there are 2 coronary arteries, but 3, 4, and even 5 are frequently present, (6) there are extensive anastomoses between branches of the major coronary arteries, (7) the *Vv. minimae Thebessi* and sinusoids are abundant in the right ventricle, particularly in the septum.

The pericardium is closely applied to the atria and major vessels of the heart but posteriorly around the ventricles and be-

TABLE 1.1

MUSCLES OF BREAST AND SHOULDER
OF THE SINGLE COMB WHITE LEGHORN CHICKEN *

Name	Origin	Insertion
M. latissimus dorsi anterior	Spinous procs. of caudal cerv. vert.	At prox. third of humerus on mid-post. surf.
M. latissimus dorsi posterior	Spinous procs. of thoracic vert.	At prox. third of humerus on mid-post surf caudal to M. lat. dor. ant.
M. latissimus dorsi metapatagialis	A muscle slip from post. end of M. lat. dor. post.	Metapatagial memb. at post. end of humeral tract.
M. rhomboideus superficialis	Under M. lat. dor. From spinous procs. of C_{14} and T_3.	Head and 3/4 of dor. margin of scapula.
M. rhomboideus profundus	Under M. rhomb. sup. From spinous procs. of C_{15} to T_6.	Distal 3/4 of med. surf. of scapula near dor. edge.
M. serratus superficialis anterior	From the vert. memb. of the T_1 rib and possibly C_{17} and T_2.	Tuber. on vent. edge of scapula, 1/4 distance from head.
M. serratus superficialis posterior	From the vert. memb. of T_2–L_2 ribs and unc. procs.	Caudal tip of scapula.
M. serratus superficialis metapagialis	From T_3 rib vent. to unc. proc.	Skin of the metapatagium.
M. serratus profundus	Vert. memb. of C_{17}–T_2 above unc. procs.	Middle 1/3 of med. surf. of scapula.
M. scapulohumeralis anterior	Vent. edge of scapula distal to glenoid facet	Posterovent. surf. of humerus, caudal to pneumatic foramen.
M. scapulohumeralis posterior	Most of lat. surf. of scapula	Posterovent. surf. of humerus on bicipital crest.
M. pectoralis thoracica **	Lat surf of keel, near vent. marg., posterolat. proc. of sternum, sternal ends of T_{2-6} and R ribs, clavicle and hypocleidium.	Ant. surf. of humerus below the distal half of the deltoid crest.
M. supracoracoideus	1. Dorsal part of lat. surf. of keel. 2. Anterolat. surf. of coracoid. 3. Vent. half of memb. on sternal notch. 4. Centr. part of sterno-coraco-clavicular memb.	Through triosseal canal to humerus: 1. Ant. surf. below deltoid crest. 2. Dorsopost. surf. at base of deltoid crest.
M. coracobrachialis anterior	Ant. surf. at dor. end of coracoid (the acrocoracoid).	Ant. surf. of humerus under ant. half of deltoid crest.
M. coracobrachialis posterior	Anterolat. surf. of coracoid and adjacent sternum.	Post. surf. of humerus on int. tuberosity.
M. sternocoracoideus	Lat. surf. of anterolat. proc. of sternum and adj. subcostal area of sternum	Middle third of coracoid on lat. half of post. surf.

tions of body parts, for example: the ilium of mammals does not extend in either direction much beyond the acetabular region, whereas in birds the ilium is extremely long. Hence the iliotrochanteric muscles are peculiar to birds and not represented in mammals or at least cannot be definitely homologized (Hudson, 1917). The ambiens muscle, which originates on the pectineal process, is peculiar to birds and reptiles and is absent in mammals. A broad, flat, triangular muscle, the iliotibialis, covers much of the lateral surface of the thigh. It is divisible into anterior, medial, and posterior parts. Gadow (Hudson, 1937) considers the anterior part as absent in mammals, the middle part as homologous with the m. tensor fasciae latae of mammals and the posterior part as homologous with the m. gluteus maximus. In birds the gastrocnemius has three parts. The external and medial parts may represent the m. gastrocnemius of man and the internal part may be homologous with the m. soleus of man.

In Fig. 1.6 is shown the superficial muscles visible from the side. The terminology for wing, limb, and girdles follows Hudson (1937) and Hudson et al. (1955, 1959, 1964), and that for head, hyoid, neck, and tail has been taken from various authors but mostly from Fisher and Goodman (1955). The names of muscles of breast, shoulder, pelvis, and thigh are presented in Tables 1 and 2 and include a brief statement of origin and insertion of each muscle.

Medial to the eye globe are two muscles in addition to the six found in mammals, i.e., m. quadratus nictitantis and m. pyramidalis nictitantis that are involved in movement of the nictitating membrane (Fig. 1.7).

The bellies of the muscles of the appendages are concentrated near their respective origins, thus shifting their weight closer to the center of gravity. The reduction of digits in the wing is a second factor in the fusion of muscle masses and their consequent multitude of origins. Centripetal placement of the muscle masses tends to produce long tendons, and in the legs especially these are guided by well-developed fibrous sheaths, loops, grooves, and canals in bone and cartilage. Sesamoid bones are common and many tendons become ossified.

Breast muscle is composed of fibers of two size groups: the large are light in color, the small are dark. In addition there are many cytochemical differences (Watzka, 1939; George and Naik, 1957, 1958, and 1959; George and Talesara, 1962).

VASCULAR SYSTEM

Heart

The avian heart has its axis in the median plane of the body. Its ventricles are nearly surrounded by the lobes of the liver. Like the mammalian heart, it is four-chambered and the circulation of blood through these chambers is similar in the two classes of vertebrates. Some of the distinctive features of the heart in birds, as found by Kern (1926), Petrén (1926), Uchiyama (1928), and Westpfahl (1961), are as follows: (1) Relative to body weight, it is heavier in birds than in mammals or reptiles, (2) neither a ductus arteriosus nor a ligamentum arteriosum is present in chickens but both structures have been reported for other species of birds, (3) a distinguishable fossa ovalis fails to persist after the closure of the foramen ovale in the embryo, (4) plaques of cartilage are normally present in the aorta at the level of the semilunar valves and sometimes in the wall of the adjacent common pulmonary artery, (5) most commonly there are 2 coronary arteries, but 3, 4, and even 5 are frequently present, (6) there are extensive anastomoses between branches of the major coronary arteries, (7) the Vv. minimae Thebessi and sinusoids are abundant in the right ventricle, particularly in the septum.

The pericardium is closely applied to the atria and major vessels of the heart but posteriorly around the ventricles and be-

TABLE 12 (continued)

Name	Origin	Insertion
M. femoritibialis externus	Lower half of femur, on lat. surf.	Patella and patellar tendon via femoritibialis medius.
M. femoritibialis medius	Most of ant. and lat. surfs. of femur betw'n trochanter and condyles.	Patella and patellar tendon.
M. femoritibialis internus	Med. surf. of the shaft of the femur.	Medial tibial crest.
M. piriformis pars caudifemoralis pars iliofemoralis	Posterolat. surf. of pygostyle. 1. Ilium caudal to ilioischiatic fenestra. 2. Caudal marg. of ischium.	Med. surf. femur ⅓ dist. distal to head.
M. semitendinosus	1. Edge of iliocaudal spine. 2. Adj. coccygeal vert.	On the ext. cnemial crest, continuing slightly distal to it.
Accessorius semitendinosi	Post. surf. of femur prox. to intercondylar furrow.	Via raphe to the belly of the semitendinosus.
M. semimembranosus	1. Vent. marg. of ischium — most of caudal half. 2. Lat. surf. of pubis opposite ischiatic proc.	Joins tendon of semitendinosus and has same insertion..
M. biceps femoris	Dorsal part of lat. wall of ilium in renal reg. above illioischiatic fenestra.	Tendon passes through biceps loop and inserts on a posterolat. tubercle of fibula ⅓ from prox. end.
M. ischiofemoralis	Most of the lat. surf. of ischium.	Lat. surf. of femur at base of trochanter.
M. obturator externus	1. Betw'n acetabulum and obturator foramen. 2. Vent. to obturator foramen.	Posterior marg. of trochanter.
M. obturator internus	Mult. heads. Inner surf. of ilium, ischium, and pubis, above and caudal to the foramen.	Lat. surf. of trochanter.
M. adductor longus et brevis, pars externa	Laterovent. marg. of ischium in 2nd quarter of marg.	Med. surf. of femur betw'n base of trochanter and the condyles.
M. adductor longus et brevis, pars interna	On pubis below acetabulum and then to laterovent. marg. of ischium to within ¼ of its caudal end.	A line on medial surf. of lower ¾ of femur.

Abbreviations:

adj. = adjacent	lat. = lateral	posteromed. = posteromedial
ant. = anterior	laterovent. = lateroventral	proc. = process
anterolat. = anterolateral	marg. = margin	prox. = proximal
betw'n = between	margs. = margins	reg. = region
dist. = distance	med. = medial	surf. = surface
ext. = external	memb. = membrane	surfs. = surfaces
iliolat. = iliolateral	mult. = multiple	vent. = ventral
inf. = inferior	posterolat. = posterolateral	vert. = vertebra and vertebrae

TABLE 1.1 *(continued)*

Name	Origin	Insertion
M. subcoracoideus	1. Dorsal lip of coracoid sulcus. 2. Sternal spine. 3. Posteromedial surf. of clavicle, near head.	With tendon of subscapularis on int. tuberosity of humerus.
M. subscapularis	1. Vent. surf. of scapula near head. 2. Med. surf. of clavicle, near head.	Int. tuberosity and capital groove of humerus.

Abbreviations:

adj. = adjacent	lat. = lateral	procs. = processes
ant. = anterior	M = musculus	prox. = proximal
anterolat. = anterolateral	marg. = margin	reg. = region
centr. = central	margs = margins	surf. = surface
C or cerv. = cervical	med = medial	T = thoracic
dor. = dorsal	memb. = membrane	tuber. = tubecosity
dorsopost. = dorsoposterior	post. = posterior	unc. = uncinate
ext. = external	posterolat. = posterolateral	vent. = ventral
int. = internal	posterovent. = posteroventral	vert. = vertebra,
L = lumbar	proc. = process	vertebrae and vertebral

* Based on dissections and notes by John A. Blair, D.V.M — Avian Anatomy Project, and terminology of Hudson and Lanzillotti (1955, 1961).

* * See Hudson and Lanzillotti (1964) for subdivisions.

TABLE 1.2

MUSCLES OF PELVIS AND THIGH
OF THE SINGLE COMB WHITE LEGHORN CHICKEN*

Name	Origin	Insertion
M. iliotrochantericus posterior	Lat. surf. of the gluteal ilium except margs, and a triangular area above the acetabulum.	Ant. marg. of the lat. surf. of the trochanter (femur).
M. iliotrochantericus anterior	Ant. ⅔ vent. marg. of gluteal ilium.	Anterolat. surf. of shaft of femur just distal to trochanter.
M. iliotrochantericus medius	Lat. surf. of gluteal ilium just ant. to the acetabulum.	Distal end of anterolat. surf. of trochanter.
M. glutaeus medius et minimus	Triangular area above and ant. to antitrochanter and below iliolat. proc.	Center of lat. surf. of trochanter.
M. iliacus	Depression ant. to pectineal proc.	Posteromed. surf. femur ⅓ dist. distal to head.
M. ambiens	Outer half of pectineal proc.	Lat. side of head of fibula via the heads of the flexor perforatus muscles.
M. sartorius	Ant. part of the crest of the ilium.	1. Med. part of patellar tendon. 2. Ant. tibial crest.
M. iliotibialis	1. Crest of the ilium caudally from origin of sartorius. 2. Dorsal surf. of ilium. 3. Iliocaudal spine. 4. Caudal marg. of ilium and ischium to ischiatic proc.	1. Patella. 2. Lat. part of patellar tendon.

[16]

; Figs. 1.14 and 1.16 and some branches m the cranial mesenteric in Fig. 1.10.

Parietal branches from the aorta supply teries to the ribs, rib spaces, vertebral ructures, and muscles through the *aa. in- rcostales, aa. lumbales, aa. sacrales lat- ales*, and *aa. coccygica laterales*. A pair large vessels in both sexes, *aa. spermati- e internae*, gives branches to the anterior bes of the kidneys and to the adrenal lands. In the female a large median ves- l, *a. ovarica*, supplies the ovary and part the oviduct, the branches of which are own in Fig. 1.16. Other lateral branches pply the remaining lobes of the kidney nd in the female, the lower oviduct. reedman and Sturkie (1963) have investi- ated in detail the arterial and venous upply to the oviduct, and have reviewed he terminology for these vessels, making ome changes. (See page 44 for additional nformation on the vascular system in the emale.)

The most posterior unpaired branch of he aorta is the *a. mesenterica caudalis*, vhich anastomoses with branches from the ranial mesenteric artery. The major ves- els to the leg are the *a. iliaca externa* and . *ischiatica externa*. The external iliac rtery gives rise to about 4 vessels includ- ng the *a. femoralis*. Most of the blood upply to the leg as well as parts of the fe- nale reproductive system comes from the xternal ischiatic artery of which there are bout 22 named branches. (See also Ni- hida, 1963.)

A short distance distal to the caudal nesenteric artery the *a. sacralis medialis* divides into a right and left *a. pudendalis interna* and a median continuation into he tail region as the *a. coccygica media*. The internal pudendal artery gives rise to a branch that supplies the internal obtu- rator muscle and a branch to the bursa and dorsal surface of the cloaca.

A large branch from the intestinal ramus of the pudendal artery parallels the side of the cloaca and enters the *corpus cavernosum* (Barkow, 1829), a body present in male chickens (Fig. 1.14) but not in fe-

males. It consists of a compact mass of capillaries with very little supporting tis- sue instead of the large vascular sinuses found in typical erectile tissue. Peripheral to these are arteries with thick muscular walls. The body has no structural connec- tion with the ejaculatory duct but its caudal tip lies beneath the round body on each side of the phallus.

We recommend that the term, *corpus cavernosum*, be changed to *glomus para- cloacalis* since Barkow's term as used by Gadow (1888) and most other writers identify this name with the large sinus- oidal erectile tissue associated with the phallus or ejaculatory duct. Nishiyama (1955) has used the term, vascular body.

Veins

A recent, comprehensive description of the veins of the chicken is not at present available. The work of Neugebauer (1845) is still a standard reference as well as the book by Gadow and Selenka (1891). Blood from the brain, deep and superficial mus- culature of the head, the ear, and the base of the skull drains by way of about 12 major vessels into the jugular veins. An anastomosis in the region of the hyoid joins the right and left jugulars. Each jugular vein receives many branches from muscle, skin, and organs of the neck by way of about 9 major vessels of which the vertebrals have the most extensive drain- age. Junction of each *v. jugularis* at the base of the neck with the *v. subclavia* forms the *vena cava anterior* (*s. v. brachiocephal- alica*) (IANC, 1956). The subclavian vein receives blood from the wing and anterior part of the thorax by way of the *v. axill- laris*, *vv. pectoris externae*, *v. coracoidea*, and *v. sternalis*. The left anterior vena cava crosses the dorsal surface of the heart to enter the right atrium at a point slightly apart from that of the right anterior vena cava.

Blood from the viscera, body wall, limbs, and tail is brought to the heart through a somewhat complicated system of channels involving two portal systems. The termi-

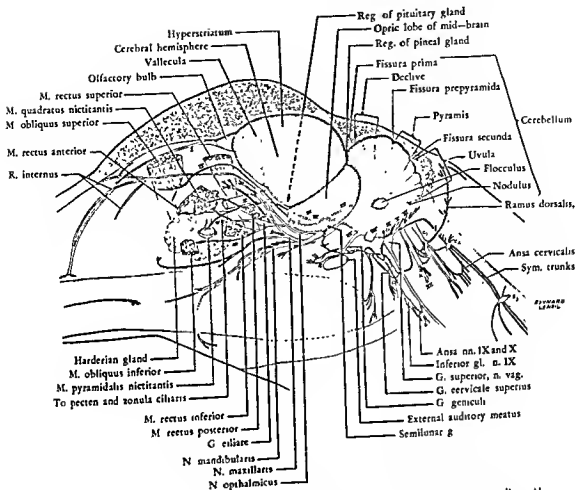FIG. 1.6 – Superficial musculature of the chicken from the left side. M., Mm., Musculus-Musculi. (From USDA)

FIG. 1.7 — Brain and cranial nerves of the chicken from the left side. G., g., ganglion; M., Musculus; N., n., nerve; R., Ramus; Reg., Region; Symp., Sympathetic; vag., vagus. (From USDA.)

yond it is drawn out into a large cone that tapers to a point within the ventral mesentery between the liver lobes.

Arteries

In the following discussion the terminology proposed by Westpfahl (1961) has been used for major arteries in the chicken. This author has given an extensive review of nomenclature.

From the base of the aorta at the level of the semilunar valves arise the right and left coronary arteries; each divides into deep and superficial branches. A pair of large vessels, the *aa. brachiocephalicae*, arise from the aorta just anterior to the

pericardial sac; each of these divides into *a. subclavia* and *a. carotis communis* (Fig. 1.17). The *a. subclavia*, in the angle between coracoid and scapula, divides into numerous branches that supply the inner and outer breast musculature and skeleton. The first branch is the *a. sternoclavicularis*, which turns ventrally, crossing the coracoid, to supply the anterior end of the sternum, the lower part of the clavicle, and the larger muscles of the breast. The subclavian then divides into *a. axillaris* and several *aa. thoracicae*. The former supplies all the wing muscles and skeleton by way of fourteen named arteries and several lesser branches. The latter supply the

sternum, ribs, and associated muscles. (See also Nishida, 1960.)

Two vessels arise from opposite sides of the common carotid artery near its point of origin, the a. oesophagica ascendens and the a. bronchialis. The former vessel sends branches to several important organs; those to the thyroid gland are the a. thyroidea cranialis and a. thyroidea caudalis. As shown in Fig. 1.17, additional branches supply the parathyroids and ultimobranchial bodies. The carotid body lies between the carotid artery and the nodose ganglion close to the cranial or to the caudal parathyroid gland (Chowdhary, 1955) and measures $0.8 \times 0.5 \times 0.5$ mm. The adventitial tissue of the small artery that enters the carotid gland has continuity with the capsule of the gland. Two types of cells are present: large, granular argyrophils and small, darkly staining, nongranular, nonargyrophilic cells. These cells extend into the tunica media of the nutrient vessel, and even into the wall of the adjacent carotid artery; the junction of these vessels forms the carotid sinus.

Each ascending esophageal artery supplies trachea, crop, and in the region of the 4th cervical vertebra anastomoses with a descending esophageal artery, from the external carotid by way of the laryngeal artery. The bronchial artery gives branches to the lower esophagus, trachea, syrinx, and primary bronchi. At the level of the thyroid gland (Fig. 1.17) arises the a. vertebralis which divides into ascending and descending branches that course through the foramina transversaria. An important branch is the a. comes nervi vagi and a lesser branch passes to the muscles and skin of ventral neck and shoulder. The carotid vessel continues forward from the level of origin of the vertebral artery along the ventral side of the cervical vertebrae. Fleming (1926) and Westpfahl (1961) along with others, name it the common carotid. Glenny (1955) and others on the basis of its embryology call it the internal carotid and Baumel (1963) has used the name dorsal carotid. The common carotid

divides at the level of the intervertebral space of the second and third vertebrae into a. carotis externa and a. carotis interna.

The external carotid has the following major branches: a. occipitalis, a. laryngica, a. auricularis, a. facialis, and a. maxillaris, each with smaller branches distributed to the structures in these named regions. The internal carotid enters the skull posterior to the external ear canal. A large branch, a. ophthalmica externa, supplies the deep musculature of the head and a small a temporalis supplies the skin. Another branch, a. alveolaris inferior, follows the mandibular nerve through the masseter muscle, supplies the palate and throat, and extends to the nasal cartilage. The a ophthalmica externa is a large branch distributed to the eyelids, nictitating membrane, retina, eye muscles, and glands of the orbital space, and to the plexus temporalis that lies in the depression of the skull above the ear opening, and according to Gadow and Selenka (1891), arises between the maxillary and mandibular branches of the trigeminal nerve.

The internal carotid gives off an a. sphenoidea before entering the cranial cavity and then supplies numerous vessels in and around the brain, some of which terminate on the upper lid and nose. Baumel (1962) found that the encephalic arteries of the pigeon were consistently asymmetric in size, arrangement, and distribution; and the illustrations by Kitoh (1962) would indicate that asymmetry was present in the chicken also.

The arteries to the viscera, legs, and tail are branches of the descending aorta. The most anterior of these, the a. coeliaca, is a median, ventral vessel that supplies blood to the esophagus, proventriculus, gizzard, liver, spleen, pancreas, and duodenum. An adjacent median branch from the aorta, a mesenterica cranialis, anastomoses with a branch of the coeliac and vascularizes about three-fourths of the intestine including the proximal ends of the caecae. The branches of the descending aorta are shown

in Figs. 1.14 and 1.16 and some branches from the cranial mesenteric in Fig. 1.10.

Parietal branches from the aorta supply arteries to the ribs, rib spaces, vertebral structures, and muscles through the *aa. intercostales, aa. lumbales, aa. sacrales laterales,* and *aa. coccygica laterales.* A pair of large vessels in both sexes, *aa. spermaticae internae,* gives branches to the anterior lobes of the kidneys and to the adrenal glands. In the female a large median vessel, *a. ovarica,* supplies the ovary and part of the oviduct, the branches of which are shown in Fig. 1.16. Other lateral branches supply the remaining lobes of the kidney and in the female, the lower oviduct. Freedman and Sturkie (1963) have investigated in detail the arterial and venous supply to the oviduct, and have reviewed the terminology for these vessels, making some changes. (See page 44 for additional information on the vascular system in the female.)

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A short distance distal to the caudal mesenteric artery the *a. sacralis medialis* divides into a right and left *a. pudendalis interna* and a median continuation into the tail region as the *a. coccygica media.* The internal pudendal artery gives rise to a branch that supplies the internal obturator muscle and a branch to the bursa and dorsal surface of the cloaca.

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males. It consists of a compact mass of capillaries with very little supporting tissue instead of the large vascular sinuses found in typical erectile tissue. Peripheral to these are arteries with thick muscular walls. The body has no structural connection with the ejaculatory duct but its caudal tip lies beneath the round body on each side of the phallus.

We recommend that the term, *corpus cavernosum,* be changed to *glomus paracloacalis* since Barkow's term as used by Gadow (1888) and most other writers identify this name with the large sinusoidal erectile tissue associated with the phallus or ejaculatory duct. Nishiyama (1955) has used the term, vascular body.

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Blood from the viscera, body wall, limbs, and tail is brought to the heart through a somewhat complicated system of channels involving two portal systems. The termi-

nology for the veins in the kidney region has been taken from Spanner (1925). (See also Portmann, 1950.) A pair of *vv. hypogastricae* (*s. vv. iliacae internae*) drain blood from the tail and caudal parts of the body. At the posterior end of the kidney they are joined by an anastomosis that receives in the mid-line the *v. coccygomesenterica* collecting blood from the terminal end of the digestive tract (Fig. 1.15).

The hypogastric, coccygomesenteric, ischiatic, and external iliac veins contribute blood to the renal portal vein (*v. renalis afferens*). Beyond the capillaries, the blood is recollected in the *v. renalis efferens*, a prominent vein on the ventral side of the kidney. Near the anterior end of the organ, the pair of efferent veins unite to form the *vena cava inferior*. A horseshoe-shaped valve intervenes between the continuation medially of the external iliac vein and the efferent vein; this valve is under the control of the autonomic system Sperber (1960) observed that blood of the renal portal system was distributed to the tubules only and the glomeruli received only arterial blood.

The posterior vena cava ascends to the heart through the right liver lobe. Before entering the right atrium it receives the *vv. hepaticae* which drain the liver. At the atrium its entrance is guarded by the right and left sino-atrial valves, which also function to close the openings from the right and left anterior venae cavae.

Lymphatic Vessels

Significant contributions to the anatomy of the lymphatic system of the pigeon and fowl have been made by Lauth (1824), Josifoff (1930), Baum (1930), and Dransfield (1944, 1915). (See Baum and Trautmann, 1933, for a brief review.) The channels associated with the major visceral arteries and veins are illustrated by Gadow and Selenka (1891) Lymph hearts, characteristic of fish, amphibia, and reptiles, may be present in some birds but are absent in most species. They have been found in chick embryos but not in adult birds.

Lymph vessels, possessing valves, are present in all classes of vertebrates but birds have fewer than mammals, and the number of vessels appears to decrease with age in birds. In the peripheral structures of the body the lymph vessels closely follow the veins or sometimes both veins and arteries; in the visceral parts of the body the lymph vessels follow the arteries but in the heart they follow neither. Lymphatic vessels are found in all parts of the body, skin, muscles, joints, bone marrow, and even in the follicles of remiges and rectrices. Only a few channels are present in the liver, and in the spleen these are limited to the capsule.

Lymph drainage from head, neck, and part of the shoulder follows the jugular veins into which vessels it empties on the right and left sides shortly anterior to the subclavian veins. Lymph drainage from the remainder of the body converges to the thoracic ducts on each side of the aorta and empties into the right and left anterior venae cavae.

Lymph nodes are generally absent in birds except that a few may be found in ducks, geese, and some other aquatic birds in the cervical and caudal regions of the body. Occasionally thymus lobes have been mistaken for lymph nodes. Plexus or rete formation along lymph channels has been found in all classes of vertebrates, although in only a few locations in mammals. These lymph plexuses in non-mammalian classes have been regarded as homologous with lymph nodes of mammals because they occur in such areas as submaxillary, axillary, inguinal, popliteal, abdominal, and similar regions (Dransfield, 1944).

The histology of lymph nodes in aquatic birds has been described by Fleury (1902), Pensa (1907), Jolly (1910), and its embryology by Further (1913). The rete mirabile of the gland is demonstrated in its development. Some have suggested that the lymphoid accumulations found concentrated to various degrees in the avian body were equivalent to mammalian lymph nodes

(Kihara and Naito, 1933; Kondo, 1937; Biggs, 1957). To Lucas (1951), Lucas *et al.* (1954), Denington and Lucas (1960), and Oakberg (1950), these are ectopic lymphoid foci, and are probably centers of reaction to extrinsic pathogenic and nonpathogenic stimuli. There are, of course, normal concentrations of lymphoid tissues such as those of thymus, spleen, cloacal bursa, and follicles within the intestinal wall. It is questionable to these authors if lymphoid accumulations are normally present in the bone marrow of the chicken, although they are so regarded by Kanesada (1956).

Limitations of space prevent a résumé of vascular histology. Morphology of blood cells in various stages of development has been presented by Lucas and Jamroz (1961).

NERVOUS SYSTEM

Central Nervous System

The same 3 meninges are present in birds as in mammals (Hansen-Pruss, 1923). Between the periosteum lining the vertebral canal and the dura mater (*pachymeninx*) are scattered epidural spaces and beneath this membrane are subdural spaces. The *leptomeninx* is composed of arachnoid membrane, arachnoid trabeculae crossing the subarachnoid space, and the pia mater. The last closely invests the brain and spinal cord and carries the vascular supply to these tissues. *Ligamentale denticulata*, present in each segment of the cord and composed of duralike connective tissue, suspend the cord transversely between dura and pia. The subarachnoid space carries the cerebrospinal fluid. The space extends caudally as far as the sinus rhomboidalis and anteriorly as far as the prosencephalon, thus spreading over the dorsal and lateral portions of the brain.

The embryonic development of the plexuses and cerebrospinal fluid has been followed in the chick from the third to the ninth day of incubation (Cohen and

Davies, 1937). The extensive thin roof of the fourth ventricle permits the passage of fluid from the cerebrospinal canal to the embryonic meningeal tissues.

The axis of the brain in relation to the axis of the skull varies a full 90° among various species of birds (Cobb, 1960; Portmann and Stingelin, 1961) and is associated with the position and size of the orbit. The brain of the chicken nearly parallels the head axis.

The ratio of the size of the olfactory bulb to the cerebral hemisphere varies from 5 to 33 per cent. The ratio for the turkey is 13 per cent. The sense of smell in birds is apparently less than in mammals and among avian species it is variable (Cobb, 1960). The bulb contains a cavity continued from the lateral ventricle of the hemisphere.

The cerebral hemispheres are considerably larger in birds than in reptiles, yet nearly all of the avian hemisphere is homologous to the basal nuclei of the mammalian brain. On the dorsal surface is a groove (vallecula) arising from the median fissure and extending laterally and posteriorly (Fig. 1.7). The sagittal elevation medial to the vallecula represents the neopallium (hyperstriatum) that perhaps is equivalent to the mammalian neocortex. A small area at the lateral surface of the avian hemisphere, called the palaeopallium, represents the well developed olfactory lobe (pyriform area) of mammals. The major cavity of the ventricle lies adjacent to the median wall of the hemisphere but in the posterior part it is a flattened cavity that extends to the lateral side of the hemisphere and at the posterior pole it separates the neostriatum from the peripheral portion. The chorioid plexus is limited to the caudo-ventral portion of the ventricle.

The diencephalon, located between the cerebral hemispheres and the laterally placed optic lobes, carries on its ventral surface the optic chiasma and the pituitary. The large thalamus is a product of the sensory alar plate, and only the small

hypothalamus is derived from the motor basal plate. The internal architecture of the diencephalon and some nuclei of the telencephalon and mesencephalon is given in detail by Huber and Crosby (1929). Recently van Tienhoven and Juhasz (1962) have prepared a brief atlas of similar centers for the chicken. Jungherr (1915) has identified the nuclei of the mesencephalon. The embryology of the corpus striatum and pallial layer has been given in detail by Haefelfinger (1958) and the evolutionary divergences by Stingelin (1958).

The morphology of the avian cerebellum has been exhaustively studied by Larsell (1948) and Larsell and Whitlock (1952) and briefly summarized by Portmann and Stingelin (1961). Some of the parts are labeled in Fig. 1.7. The medulla of birds is well developed in the vestibular region, and associated with this is a well-developed Deiter's nucleus receiving fibers from the cerebellum. Pontine fibers and pontine nuclei are well developed also. The development of the hypoglossal nerve and its associated nuclei is related to the utilization of the tongue; thus it varies among different species of birds.

The spinal cord lies within the neural canal of the vertebral column and extends from the medulla to the last free vertebra of the tail. The embryonic retraction of the spinal cord, characteristic of mammals, does not occur in birds and there is no cauda equina. Huber (1936) who has described the internal and external features of the cord in the pigeon lists in that species 39 pairs of spinal nerves. We identify approximately 42 in the chicken. Enlargements of the cord are present in the regions of the plexuses—brachial, lumbar, ischiatic, and pudendal (Goller, 1962).

The intumescentia lumbalis (s. glycogen body) fills a rhomboid sinus in the dorsal half of the spinal cord in the area of the ischiatic plexus. It is a structure peculiar to birds. The details of its development have been reported by Ganfini (1950) and De Gennaro (1959) as observed in the chicken embryo during the seventh to the thirteenth day of incubation. There is first an invasion of meningeal vessels into the ependymal cone, then a swelling of the cells and the body expands laterally against the neural portion of the cord. The leptomeninx lies outside the sinus rhomboidalis. The spinal canal remains closed beneath the glycogen body. Therefore the sinus does not represent a failure of the embryonic neural ridges to close but is rather a specialized modification of the cells of the dorsal ependyma and alar plate. Dickson and Millen (1957) determined that the glycogen body is partly intrapial and partly subpial; the latter portion surrounds the central canal.

A marginal paragriseal column extends most of the length beneath the lateral surface of the cord and is visible grossly in the region of the ischiatic plexus.

Cranial and Spinal Nerves

Birds have 12 cranial nerves as do mammals; the existence of an accessory nerve in birds has been questioned by some (Van Tyne and Berger, 1959) but our dissections (Fig. 1.7) agree with those of Cords (1904). N. olfactorius (I) arises from the olfactory bulb and terminates in the superior concha. N. opticus (II) arises from the optic chiasma beneath the thalamus and passes laterally to enter the medioventral part of the optic globe. N. oculomotorius (III) arises from the mesencephalon close to the mid-line and innervates the superior, anterior, and inferior recti and the inferior oblique muscles. The ciliary ganglion, located in the angle between superior and inferior rami of the oculomotor nerve, innervates the pecten, and the ciliary zone. The ganglion receives a branch from the ophthalmic nerve of the trigeminal. (See Carpenter, 1906, for details of the development of nerves in this area.)

The small n. trochlearis (IV) arises from the dorsal side of the brain and passes down between the optic lobe and the cerebellum to innervate the superior oblique muscle. Cords (1904) describes a communi-

cating branch with the trigeminal that we did not observe.

The origin of n. trigeminus (V) from the floor of the brain as well as the semilunar ganglion is partly covered by the optic lobe. The ophthalmic nerve, the first branch of the trigeminal, extends forward along the ventral edge of the cerebral hemispheres and thence laterally along the superior rectus and the superior oblique muscles, arriving at the dorsal part of the nasal septum. Many branches are distributed to the nasal area.

The maxillary nerve of the trigeminal has many branches, some to the region of the temporal plexus (p. 20), a branch above the eye to the nasal region, and a branch below to palatine and maxillary regions. The third branch of the trigeminal, the mandibular nerve, innervates many muscles and glands of the jaws and face and carries afferent fibers. (See Cords, 1904; Lakjer, 1926; Starck and Barnikol, 1954, for details).

N. abducens (VI) arises from the floor of the medulla, near the mid-line between the origins of the right and left trigeminals, and innervates the posterior rectus, the quadratus, and the pyramidalis muscles. The n. facialis (VII) also arises from the floor of the medulla beneath the flocculus. It extends a short distance to the dorsal margin of the ear where it divides into its two main branches, at which point is a small geniculate ganglion. An anastomosing network is present above the ear from which an anastomotic branch extends to the semilunar ganglion. We are uncertain of the identification of the chorda tympani nerve; our dissections did not agree in all details with those of Cords (1904) and Yntema (1944). Smith (1941) failed to find any branch joining the fifth and seventh nerves in the turkey.

We were unable to obtain a good dissection of the n. acustica (VIII) and in Fig. 1.7 it is shown as a short stump. The nerve arises from the side of the medulla dorsal to the origin of the seventh nerve. Cords (1904) observed a ramus anterior

and ramus posterior; the former sending branches to utricle, lateral and anterior ampullae, and the latter, to the posterior ampulla, sacculus, and cochlea.

N. glossopharyngeus (IX), n. vagus (X), and n. accessorius (XI) arise as a group of rootlets from the side of the medulla. The glossopharyngeal emerges from the anterior edge of the superior ganglion of the vagus. The accessory nerve is embedded on the opposite side of the same ganglion. The ninth nerve parallels the vagus caudally and ventrally. Beneath the hyoid there is a conspicuous anastomosis, immediately proximal to which is the inferior (r. petrosal) ganglion of the ninth nerve. About midway between the origin of the ninth nerve and its ganglion is the superior cervical sympathetic ganglion, with branches forward to the facial nerve, and ventrally to form a plexus around the carotid artery. It also sends an anastomosis to the hypoglossal nerve and presumably continues within the network between the twelfth cranial and first cervical nerves, to contribute to the sympathetic trunks on each side of the spinal cord.

The distribution of the vagus along the neck and to the visceral organs has been described by Watanabe (1960) in the fowl and Malinovsky (1962, 1963) in the pigeon. In the region of the parathyroid glands there is located the nodose ganglion (Fig. 1.17). The loops of the recurrent laryngeal nerves include the pulmonary artery on the right but not on the left.

The accessory nerve has been variously described. The arrangement shown in Fig. 1.7 agrees with that indicated by Watanabe (1960). The n. hypoglossus (XII) arises from the motor area of the medulla by two roots (Cords, 1904; and illustrated in Hughes, 1934–35) and gives branches to larynx, trachea, tongue, and hyoid in addition to anastomoses with adjoining nerves. Smith (1941) states that in the turkey, the hypoglossal is fused with a branch of the glossopharyngeal nerve.

The first spinal nerve emerges between the skull and atlas (Huber, 1936; Goller,

1962) and thus each succeeding spinal nerve issues from the vertebral canal through the intervertebral foramen. Each spinal nerve lies anterior to its same numbered vertebra. The first cervical nerve lies within the cranial cavity and emerges through the *foramen magnum*. It innervates most of the neck musculature adjacent to the head. The remaining cervical nerves are small as far as the level of the brachial plexus. This plexus is composed of cervicals 15 through 17 and thoracic 1, a total of 4 nerves. Yasuda (1960) and Goller (1962) show nerves, $C_{13\,16}$, and Howell (1937) shows nerves $C_{14\text{-}17}$, involved in the plexus in the chicken. Variability in plexuses has been carefully studied by Furbringer (1888), Gadow and Selenka (1891), and recently by Baumel (1958).

In the pelvic region the *plexus lumbalis (s. cruralis)* includes the third and fourth lumbar and first sacral nerves Goller (1962) shows 4 nerves in this plexus. The *plexus ischiaticus (s. sacralis)* is composed of a caudal ramus from the first sacral nerve and nerves from sacrals 2 through 5. Goller (1962) shows that 6 spinal nerves are involved. Boas (1933) has illustrated the variability in the lumbosacral plexus for 5 chickens. The *plexus pudendalis* is usually depicted as composed of nerves from the first two of the caudal vertebrae as well as a caudal ramus from the last sacral (Goller [1962] shows 4 roots) but all of the caudal and coccygeal nerves are joined by anastomoses that become concentrated into 4 nerve trunks so that it would be difficult to establish a sharp functional separation from the pudendal plexus and the remainder of the caudal nerves (Boas, 1933). Baumel (personal communication) states that in the pigeon a coccygeal nerve plexus can be distinguished posterior to the pudendalis plexus.

Terminology for the nerves distributed to the wing and leg of the fowl has been reviewed by Buchholz (1959-60). (See also Gadow and Selenka, 1891; Yasuda, 1960-61; Fisher, 1946; Hudson and Lanzillotti, 1955.)

Autonomic Nervous System

Two sets of fibers, acting complementary to one another, form the parasympathetic and the sympathetic components of the autonomic nervous system. Portmann and Stingelin (1961) have given a useful diagram of the bird's autonomic system; the relation of the sympathetic trunk to the cranial and spinal nerves is shown by Huber (1936) for the pigeon. Hammond and Yntema (1947, 1958) and Yntema and Hammond (1945) have given detailed reconstructions of the autonomic system in the chick embryo and in the hatched chick 8 days old. Hsieh (1951) has described in great detail the anatomy of the sympathetic and parasympathetic nervous system of the fowl, tracing the small branches to the various organs of the body. Included is an extensive coverage of muscles, arteries, and veins. A brief summarization is given by Grahame (1955).

The sympathetic trunk begins at the superior cervical ganglion (Fig. 1.7) and extends to the level of the sixth coccygeal vertebra. Hsieh (1951) counted 57 pairs of ganglia. The interganglionic cord divides to pass above and below the transverse processes of those vertebrae bearing ribs. The splitting of the cord is more conspicuous in the anterior end of the rib series than caudally. The cord in the mid-body region (T_1–L_1) gives rise to well developed branches distributed by way of the mesenteries to visceral organs. Hsieh found that communicating rami were absent except in the anterior ganglion and last two cervical ganglia and in the caudal part of the body. He found that usually the ventral ramus of each spinal nerve ran through a notch on the dorsal and lateral faces of its sympathetic ganglion and received sympathetic fibers directly.

Sensory Receptors

The largest sensory end-organs, the Vater-Pacini corpuscles, are present near the follicles of contour feathers and in non-feathered areas (Winkelmann and Myers,

1961). These lamellar structures are very numerous in the dorsal dermal layer of the claw and in the beak, especially of ducks. They are present around the joints and between the tibia and fibula (Schildmacher, 1931), in the hard palate, and in the eyelids. Winkelmann (1960) recommends that the synonyms, Herbst, Rauber, and Leydig corpuscles, be dropped because these are all identical structures.   Schildmacher (1931) has identified the layers of a Vater-Pacini corpuscle from the central nerve outward as inner bulb (s. granular layer), inner lamellar layer, outer lamellar layer, and connective tissue capsule. He has suggested that these corpuscles are vibration receptors.

The Grandry corpuscle is characterized by having 2 or more cells, oval in shape and flattened. Their central surfaces are slightly concave and within this space is a tactile disc that receives the terminal network of the axon. Each of these tactile bodies is supplied by a myelinated nerve. These corpuscles are common in the beak of ducks (Portmann, 1961a).

Taste buds are not concentrated on specialized papillae as in mammals but are distributed as isolated follicles on the base of the tongue and floor of the pharynx (Lindenmaier and Kare, 1959; Moore and Elliott, 1946). Duncan (1960) pointed out that birds have fewer than one hundred taste buds whereas mammals have many thousands. The details of structure are shown by Portmann (1961a) who considers that the chief difference from a mammalian taste bud follicle is a peripheral concentration of small sheath cells, but such a sheath was not seen by Greschik (1917) in an Amazon parrot. Avian taste buds may be composed of a few cells that merge with the epithelial cells or may be distinct groups of cells often associated with ducts of palatine glands (Botezat, 1910).

The olfactory nerves from the posterior concha of the pigeon and chicken are a discrete bundle provided with a connective tissue sheath (Locatelli, 1927) but in vultures, albatrosses, and oilbirds, that are sensitive to dilute odors, there are a multitude of delicate strands beneath the olfactory epithelium (Bang, 1960).

Special Sense Organs

The eyes of birds are large relative to the size of the head, yet without certain structural modifications as discussed below, they would need to be even larger to function as effectively as they do. Cones are much more abundant per unit of retina (outside the fovea) of birds than mammals, and since the ratio of efferent nerve fibers is approximately 1:1 for the cones and rods, the optic nerves of birds are relatively large. Pumphrey (1961a) has pointed out that the visual acuity in the fovea is about the same in birds as in mammals but that birds have a much greater range of accommodation, and because cones are more numerous outside the fovea, birds can maintain an accurate view of a wide field whereas the sharpest field for man is limited to 2.5°.

The fovea of birds lies in an *area centralis*. It may be a circular elevated area surrounding the fovea or it may be a streak across the retina in a horizontal plane. The latter is found mostly in species having an aquatic habitat. The concentration of cones in the area is even greater than in the fovea. A comparative study of the ocular fundus of most orders of birds has been given by Wood (1917).

The pecten in birds is a black vertical fan or comb that overlies the optic nerve. Its shape is variable among different species of birds. In the owl it has the form of a trapezoid; its base is 8 mm. and its free edge 5 mm. long. It carries numerous vessels and is thrown into 5 to 30 folds. Details of structure are given by Seaman and Storm (1963) and Seaman and Himelfarb (1963). It has been suggested that the pecten functions as a screen to reduce the light that would enter both eyes simultaneously and in this way helps to retain monocular vision.

The ear of birds is composed of three

parts, an external auditory canal, a middle ear crossed by a columella, and an inner ear divided into a superior part with semicircular canals and utriculus, and an inferior part composed of sacculus, cochlea, and lagena.

Birds lack an external auricle. The external opening is covered with roarse feathers that probably reduce the noise of air movements across the canal and exclude insects. A tympanum forms the boundary between outer and middle ears. Implanted on its inner surface is the columella, a trumpet-shaped bone. The discshaped foot of the stapes (columella) fills the space of the oval window and adjacent to it is the round window that also is covered by a membrane that retains the endolymph fluid. The oval and round windows respond reciprocally to the vibrations transmitted through the stapes to the oval window. Pressures within the middle ear are equalized through the auditory canal. Air cells in some of the head bones also connect with the middle ear cavity.

The utriculus is a sac-shaped structure into which open the three semicircular canals, the horizontal canal has its ampulla with its sensory crest at the anterior end. Joining it is the ampulla for the anterior canal, which generally is the largest and which caudally unites with one end of the posterior vertical canal. The latter has its ampulla near the sacculus. Within the sacculus are flattened sensory discs, *macula utriculi* and *macula neglecta*; the latter is absent in mammals.

The sacculus, located at a 40° angle inferior to the utriculus, is joined to it by a short duct. From it arises a short endolymphatic duct that penetrates the dura of the brain cavity and terminates as the endolymphatic sac. Arising from the sacculus is a slightly curved finger-shaped structure, the cochlea, which is not coiled as in mammals but has the same three canals, *scala vestibuli*, *scala media*, and *scala tympani*. The tegmental membrane is cellular and is thrown into several folds. The basilar membrane probably functions

in the same way as in mammals but would appear to have a more limited range (see Pumphrey, 1961b). The lagena is a static organ and at its tip is the *macula lagenae* functioning as does the *macula sacculi*. Measurements of length and volume for the inner ear of the chicken have been presented recently by Watabe (1960).

RESPIRATORY SYSTEM

Nose, Pharynx, and Trachea

The nose occupies a triangular space between external nares and the margin of the eye. Between the nose and the integument are the lacrimal sinuses that empty into the nasal cavity through the lateral wall. A septum divides the pair of nasal cavities. The lateral walls of the cavity have three conchae, anterior (squamous), medial (ciliated), and posterior (olfactory). (For the anatomy of the respiratory system of the turkey, see Cover, 1953a, b, c.)

Mucosal glands of the lateral and septal walls are arranged in rows separated by ciliated cells (Bang, 1961). The ciliary currents follow the rows; there is a spiral path across the middle concha, and from the middle meatus the direction of flow is toward the roof of the pharynx. The pattern of ciliary currents on the septum corresponds approximately to that on the lateral wall. When young chickens are deprived of water the mucous flow becomes sluggish and erratic (Bang and Bang, 1961). The mucus on the nonciliated olfactory epithelium is removed by the traction from the ciliary activity that surrounds the olfactory area.

The nasal gland (s. salt gland) is small in the galliformes and lies lengthwise against the roof of the orbital cavity. Functionally it supplements the kidneys by reducing the electrolyte content of body fluids when the level is higher than can be handled by the kidneys. It has a particularly important function in marine birds where the salt load is especially great. In its embryology the gland arises from the nasal cavity. It extends forward under the

maxillary process of the nasal bone and its duct empties into the middle meatus through a narrow slit (Technau, 1936). In marine birds the gland usually lies on the dorsal surface of the frontal bone near its lateral margin as shown by Fänge *et al.* (1958) for the herring gull. The organ is divided into lobes and each of these lobes has secreting tubules arranged around a central canal. The organ is supplied by branches from both internal and external ophthalmic arteries whose capillaries have the radial pattern of the tubules.

Scothorne (1959) determined in the domestic duck that the cytoplasm of the secretory cells was densely packed with mitochondria and that the secretion produced was neither mucoid or serozymogenic. Doyle (1960), using the electron microscope, observed that the secretory cells of the salt gland in the black-backed gull (*Larus marinus*) were packed with narrow canals oriented between base and apex of the cell. A salt load caused these spaces to enlarge.

The palate is in part soft and in part hard. Between each right and left half is a longitudinal slit that provides a passageway from nasal to oral cavity (Fig. 1.10). The anterior part of the pharynx begins between the choana and the common opening for the auditory tubes. The entire base of the tongue is located within the pharynx, whereas the tip lies within the mouth cavity. Behind the base of the tongue is the *rima glottidis* or opening of the upper larynx. An epiglottis is absent in birds. The shape of the upper larynx is maintained by the cricoid and arytenoid cartilages—a thyroid cartilage is absent in birds. The opening leads to a tubular trachea supported by complete cartilage rings. Half of each ring is wide and half narrow. These wide and narrow parts alternate with those of adjacent rings.

Immediately above and below the bifurcation of the trachea is a modified area that form a *syrinx* or lower larynx. There is a pair of external tympanic membranes on the lateral surfaces of the syrinx and a pair of internal tympanic membranes on the medial surfaces of the primary bronchi in the region of the pessulus. The syrinx is composed of 4 groups of skeletal elements, namely the 4 tracheal rings above the syrinx proper, the first 3 bronchial half-rings below the bifurcation, 4 intermediate syringeal cartilages located between the two groups mentioned, and finally a bony pessulus (Myers, 1917). In many species of ducks there is an enlargement of the syrinx in males, the tracheal bulla.

Three pairs of striated muscles support the upper larynx and trachea: (1) the combined thyroglossus and the thyrohyoideus from the dorsal surface of the basilyal to the ventral surface of the cricoid cartilage, (2) the tracheohyoideus that extends as bands along the side of the trachea from the sternal spine to the posterior end of the cricoid cartilage, and (3) the sternotrachealis from the medial surface of the anterolateral spine of the sternum along each side of the trachea to the upper larynx (Myers, 1917).

Lungs and Air Sacs

Air is brought to each lung by a primary bronchus that continues through the length of the lung as an S-shaped tube, the mesobronchus, and ends in an abdominal air sac. Secondary bronchi arise in 4 groups from each mesobronchus, 4 ventromedial, 6 dorsomedial, 6 lateral, and about 20 dorsal. The parabronchi (tertiary bronchi) are in the form of loops and usually join an anterior and a posterior group of secondary bronchi (Juillet, 1912; Locy and Larsell, 1916; King, 1956). Air capillaries pass radially from the lumen of the parabronchus and intermingle with the blood capillaries which unite interlobular pulmonary arteries and veins.

An extensive system of air sacs is peculiar to birds (Fig. 1.8). These are thin-walled outpocketings from the bronchi of the lungs that extend around the visceral organs and into many of the bones. The bones of the skull contain pneumatic cav-
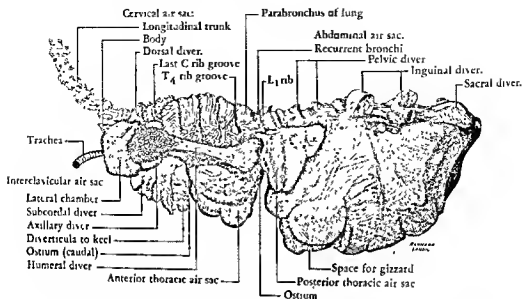
FIG. 1.8 — Latex cast of the lungs and air sacs of a chicken from the left side. The cervical air sac in the neck extends anteriorly as far as the atlas, but only that portion is shown here from the 12th cervical vertebra caudally. C, cervical; diver., diverticulum; L, lumbar; T, thoracic. (From USDA.)

ities but these are not connected to the air sacs. These develop as outgrowths of nasal and middle ear cavities. Six pairs of air sacs arise in the chick embryo from the secondary bronchi and the last pair from the caudal end of each mesobronchus. During development there is a fusion of the second and third pairs both across the mid-line and anteroposteriorly to form the large interclavicular air sac (Locy and Larsell, 1916). This air sac has about three primary chambers and several secondary outpocketings or diverticula; the most extensive is a group of axillary diverticula around the shoulder joint lying between the pectoral and supracoracoid muscles. The humeral diverticulum arises from one of these by a narrow canal to enter the proximal end of this bone through the pneumatic foramen. Other diverticula extend into the keel of the sternum, the clavicles, and coracoids. The interclavicular air sac lies entirely below the esophagus. (See Müller, 1908, for the air sacs of the pigeon.)

The most anterior air sac, the cervical, lies above the esophagus and the main chambers fuse across the median line. Four longitudinal trunks extend forward with many cross connections in and around all of the cervical vertebrae except the first. Diverticula penetrate the anterior thoracic vertebrae also and the vertebral members of the first and second thoracic ribs. (See also King, 1957.)

The anterior and posterior pairs of thoracic air sacs are located ventrally and caudally to the lungs and have no diverticula. The turkey has only one pair of air sacs in the thoracic region (Cover, 1953c; Lucas and Denington, 1961). The abdominals form the largest pair of air sacs and invest the pancreas, duodenum, and the coils of the intestine, the caeca, and gonads and the left abdominal air sac surrounds about half the gizzard. Two diverticula surround the hip joint, but none enter the femur, although they do in some species of birds such as the ring-necked pheasant and the Cooper's hawk. Diverticula from the dor-
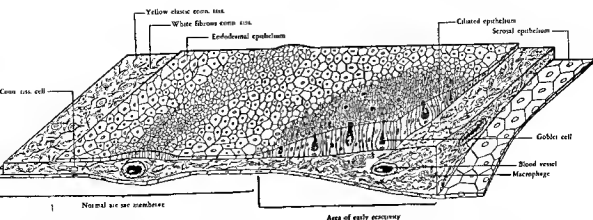
FIG. 1.9 — Stereogram of abdominal air sac wall of the chicken in the vascular part. Conn. tiss., Connective tissue. (From USDA.)

sal margin practically surround the kidneys and extend into the foramina of the lumbar and sacral vertebrae as well as inside these vertebrae and the iliac bones. The thoracic vertebrae and all of the coccygeal vertebrae as well as the pygostyle are not pneumatized. It has been claimed that the circulation of air through the abdominal air sac reduces the temperature of the testis by a few degrees but Herin et al. (1960) have shown that this is not the case.

A large part of the abdominal air sac is free from adjacent muscle and body wall tissues and therefore is suitable for histologic study (Fig. 1.9). A healthy membrane is extremely thin yet is composed of three layers, an inner simple squamous, endodermal epithelium, a middle splanchnodermal connective tissue layer, and an outer, mesodermal simple squamous serosal epithelium. The vascular and nerve supplies enter the connective tissue layer from the dorsal and lateral body wall. The ventral wall of the abdominal air sac is avascular. Collagenic and elastic fibers form the framework for the middle layer; reticular tissue is absent except perhaps in the blood vessel walls. The endodermal epithelium may sometimes transform into a simple cuboidal or columnar ciliated epithelium within which develop unicellular mucous glands. It is thought that the

ciliated epithelium develops in response to low grade stimuli.

DIGESTIVE SYSTEM

Oropharynx

The oropharynx extends from the tip of the beak to the laryngeal eminence. The junction between the embryonic mouth cavity and visceral arches has been selected as the boundary between mouth and pharynx. This boundary, that has been indicated by dotted lines in Fig. 1.10, cuts across the tongue at the level of the paraglossobasihyal joint. On the roof, the corresponding boundary lies between internal choana and auditory tubes. From these points the lines extend to the angles of the jaws.

The epidermis of the beak has produced a keratinized corneum, which is rigid in most birds but is soft in anserines. The mid-dorsal line of the upper beak is the culmen; the cutting edge, the tomium; and the line of fusion of the two halves of the lower jaw, the gonys. The upper beak extends backward around the external nasal opening and along the maxilla, producing a nasal notch between these processes. About midway along the length of the jaws is located the caudal margin of the gape, the soft tissues of which form the rictus.

Heidrich (1908) describes for the chicken

5 transverse rows of palatine papillae (also shown by Calhoun, 1954) in the roof of the mouth, the last row of which is the largest. In addition, numerous smaller hard tubercles are present between the rows. At the anterior end and along the margins of the mouth are a medial and a pair of longitudinal ridges.

The oropharyngeal glands are small and number at least one hundred. Some of these are illustrated by Heidrich (1908) and Fahrenholz (1937). Calhoun (1954) has followed the classification of Schauder (1923) which is summarized as follows:

1. Glands on the floor of the oral cavity.
   a. Anterior submaxillary.
   b. Posterior submaxillary.
2. Gland in the angle of the mouth (rictus).
   a. Angular oral gland.
3. Glands of the tongue.
   a. Anterior lingual.
   b. Posterior lingual.
4. Glands of the roof of the mouth.
   a. Maxillary gland (Heidrich, 1908).
   b. Medial and lateral palatine glands.
   c. Sphenopterygoid.
5. Glands of the pharyngeal canal.
   a. Crico-arytaenoideae.

All of the glands are compound tubular and produce only mucus.

Esophagus

The esophagus extends down the right side of the neck, enters the thoracic inlet, and follows the mid-line above the trachea to the proventriculus. Many large compound tubular glands empty into the lumen of the esophagus especially in its upper part, and numerous lymphoid follicles are present in its walls. The crop arises as a diverticulum from the esophagus and is positioned lateral to this tube immediately in front of the thoracic inlet. The greater curvature is large when the crop is filled. The relatively smooth mucosa characteristic of the esophagus extends through that portion of the crop forming the lesser curvature. In most birds the crop is a vessel that only stores food temporarily but in pigeons and doves it has the additional function of secreting "crop milk" used in feeding the young.

Stomach

The stomach of birds has two parts, proventriculus (s. glandular stomach) and gizzard (s. muscular stomach). The former is a highly glandular, spindle-shaped organ constricted slightly at its cephalic and caudal ends (Figs. 1.10, 1.11A,B). The mucosa is thrown into plicae and contains many tubular glands (Calhoun, 1954). The surface is dotted with crater-shaped elevations that are the openings for the deep proventricular glands (Fig. 1.11B). The plicae are covered with a simple columnar epithelium that contains goblet cells. The numerous deep proventricular glands are arranged radially around the cavity of the glandular stomach. These glands are compound tubular and the terminal secretory parts empty into a large common cavity within each lobule.

In galliform birds the gizzard is particularly well developed; a comparative study has been presented by Pernkopf (1937). The translocation of the pyloric exit adjacent to the isthmus of the stomach is found in many vertebrates; this produces a short lesser curvature in the chicken and the entire periphery of the muscular stomach becomes the greater curvature. The mantle of muscles on the two faces of the gizzard has a radial arrangement. These lateral muscles (mm. laterales) are united in the center to the central tendon (s. facies lata tendina, tendinous aponeurosis), and along the dorsal and ventral edges of the greater curvature are the semiannular faces. The muscles form two layers, an inner and an outer (Fig. 1.12). Caudal to the isthmus is an intermediate part and at the opposite pole is a thinwalled, caudal sac. The walls of both these are supported by intermediate muscles (mm. intermedia).

In the chicken there is no distinct endpiece at the exit from the gizzard as in

many birds, nor a constriction between end-piece and duodenum called the pylorus. Calhoun (1954) found Brünner's glands (characteristic of the transition between pyloric stomach and duodenum in mammals) in the intestine adjacent to the gizzard. Rosenberg (1931) did not find such glands in the turkey.
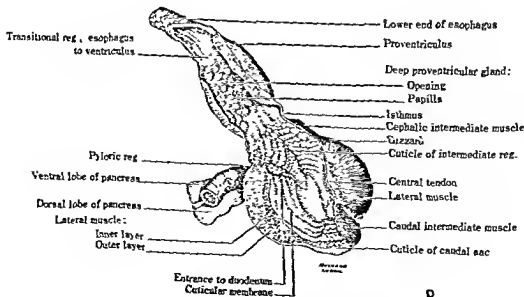
Calhoun (1954) and Pernkopf and Lehner (1937) have noted the loss of the *muscularis mucosa* beneath the cuticle of the gizzard. A few fibers noted in our preparation are shown in Fig. 1.12; therefore the *tunica propria* is around the glands and immediately below them, and *the submucosa is adjacent to the lamina muscularis.*

The thick cuticular lining of the gizzard
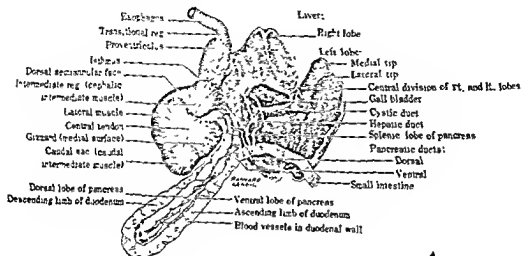
A

B

is an avian characteristic. Its histologic structure has been analyzed by Eglitis and Knouff (1962). The simple tubular glands of the mucosa secrete within their lumina stiff vertical rods that are maintained intact to the free surface of the cuticle; the epithelial surface as well as the gland crypts secrete a less rigid matrix that surrounds the rods and forms horizontal striae or lamellae. Incorporated within this matrix are desquamated cells.

Intestine and Caeco

The intestine is divided into duodenum, small intestine, and rectum. At the junction of the latter two parts arises a pair of caeca. Gadow (1879, 1889), Mitchell (1901), and Beddard (1911) have applied the coiling of the intestine to taxonomic problems; the coiling in the chicken is one of the most simple among the birds.

The duodenum with its descending and ascending limbs encloses the dorsal and ventral lobes of the pancreas. The pancreas is held between the two layers of the mesoduodenal omentum (Fig. 1.10). The microscopic structure of the turkey duodenum (Rosenberg, 1941) is representative of the galliforms; the villi are flattened plates and are arranged in a herringbone pattern (Fig. 1.11C). The pattern varies in different parts of the digestive tract and

in different species of birds (Müller, 1922). The phylogeny of folds and various types of villi is discussed by Clara (1927). The epithelium is simple columnar and dips into secretory crypts; the supporting tissue forms the *tunica propria* and its peripheral boundary is the *muscularis mucosa*. The submucosa is poorly developed and the muscular stratum is composed of an inner circular layer, a wide circular but slightly oblique layer and an outer longitudinal layer against a subserosa, covered by a simple squamous serous membrane. Calhoun (1954) has described the variations of this pattern for different parts of the digestive tract of the chicken, and Malewitz and Calhoun (1958) for the turkey.

The small intestine, suspended by the dorsal mesentery, pivots around a single point of attachment, at which point the intestine penetrates the mesentery in a dorsal direction. From the straight portion of the lower intestine and rectum arises a pair of caeca; these extend forward, curve to the right and turn caudally where they terminate as closed pouches.

The caeca have diverse forms among species of birds; they are moderately long in the chicken. In pigeons, hawks, eagles, vultures, and parrots as well as some passerines, caeca are rudimentary or absent (Gadow and Selenka, 1891). All of the in-
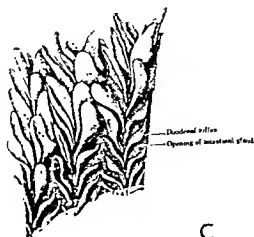
C

FIG. 1.11 — Chicken:

A. Dorsal view of liver, duodenum, pancreas, gizzard, and proventriculus showing particularly the bile and pancreatic ducts. lt., left; reg., region; rt., right. (From USDA.)

B. View showing the interior of the esophagus, proventriculus, and gizzard. reg., region. (From USDA.)
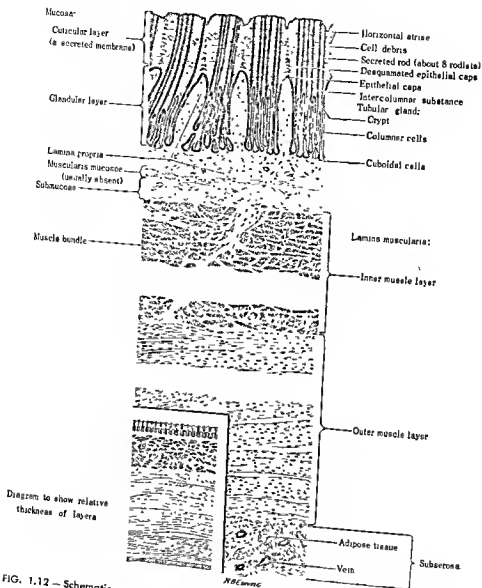
C. Villi of duodenum. (From USDA.)

FIG. 1.12 — Schematic cross-section showing the histology of the layers of the wall. (From USDA.)
Insert shows the relative thickness of the layers of the wall. (From USDA.)
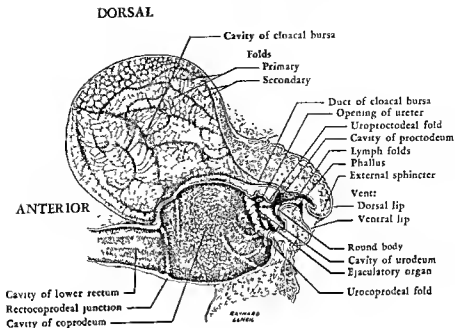
testine between the caecal junction and the cloaca constitutes the rectum.

Cloaca

The cloaca has three chambers: (1) the coprodeum (fecal chamber) that receives the rectum, (2) the urodeum (urogenital chamber) that receives openings from the ureters and the male or female reproduc-tive ducts, and (3) the proctodeum (vesti-bule) into which empties the duct from the cloacal bursa (s. bursa of Fabricius). There are four folds separating the com-partments (Fig. 1.13): (1) a slight thicken-ing of the wall between the rectum and coprodeum, (2) a well-developed membrane between coprodeum and urodeum, (3) a fleshy membrane between urodeum and

DORSAL

FIG. 1.13 — Midsagittal section of cloaca and cloacal bursa of chicken showing the interior of the right wall. m., muscle. (From USDA.)

ANTERIOR

- Cavity of cloacal bursa
- Folds
  - Primary
  - Secondary
- Duct of cloacal bursa
- Opening of ureter
- Uroproctodeal fold
- Cavity of proctodeum
- Lymph folds
- Phallus
- External sphincter
- Vent:
  - Dorsal lip
  - Ventral lip
- Round body
- Cavity of urodeum
- Ejaculatory organ
- Urocoprodeal fold

Cavity of lower rectum
Rectocoprodeal junction
Cavity of coprodeum

proctodeum, and (4) a membrane at the caudal end of the proctodeum that under conditions of inactivity is folded transversely so that the dorsal and ventral halves face each other. A description of the male accessory organs is given on page 42.

Liver and Pancreas

The liver is a bilobed structure lying ventrally to the posthepatic membrane (s. transverse membrane) and is located within a closed hepatic cavity (Beddard, 1896, 1898; Butler, 1889). The lobes are separated by a ventral mesentery. The right lobe is the larger and the left is partially divided. The shape and variability of these lobes have been analyzed by Lucas and Denington (1956). On the dorsal surface both lobes have central divisions (Fig. 1.11A).

Most species of birds have a gallbladder but it is absent in the hummingbirds and in some genera of pigeons, parrots, cuckoos, and woodpeckers (Van Tyne and Berger, 1959). In the chicken there are two bile ducts, the hepatic arising directly from the liver, and the cystic arising from the gallbladder. Their point of entrance marks the separation between the duo-

denum and the small intestine.

The elongated pancreas in the chicken occupies the duodenal loop. The pancreas in the embryo develops from 3 endodermal buds to form a dorsal lobe and a right and left ventral lobe (Hamilton, 1952; Romanoff, 1960). The ducts and lobes shift position during development; the ventral ducts come to lie close to the hepatic ducts and the dorsal duct is somewhat removed. Three ducts from the pancreatic lobes to the duodenum are shown in Fig. 1.11A. According to Romanoff (1960), the right ventral pancreas fuses with the dorsal pancreas and the left fuses with the right ventral pancreas and ultimately with the dorsal lobe. Dorsal and ventral as labelled in Figs. 1.10 and 1.11A and B refer to their position in the duodenal loop, rather than to their embryonic origin.

The splenic lobe is a continuation of the dorsal lobe and usually lies across the surface of the spleen. The cross-sectional area of the pancreas is greatest toward the distal end (Lucas et al., 1954). The pancreatic islets contain either alpha or beta cells in conjunction with delta cells. Alpha islets are largest and are most abundant in the splenic lobe. Intranuclear inclusions were
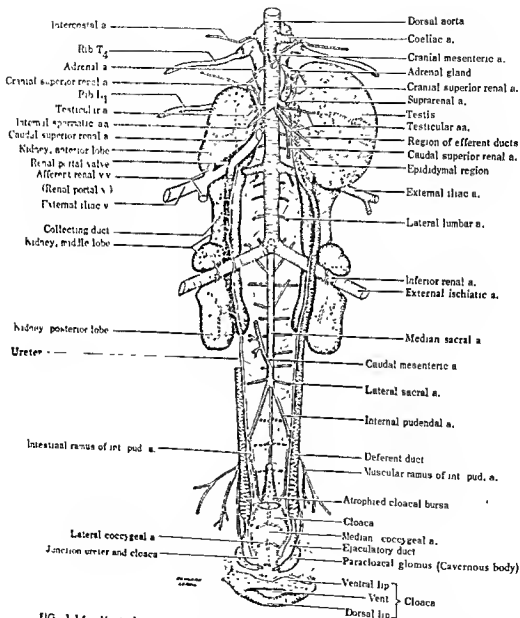
Labels on left side (top to bottom):
Intercostal a.
Rib T₇
Adrenal a.
Cranial superior renal a.
Rib L₁
Testicular a.
Internal spermatic aa.
Caudal superior renal a.
Kidney, anterior lobe
Renal portal valve
Afferent renal vv.
(Renal portal v.)
External iliac v.
Collecting duct
Kidney, middle lobe
Kidney posterior lobe
Ureter
Intestinal ramus of int. pud. a.
Lateral coccygeal a.
Junction ureter and cloaca

Labels on right side (top to bottom):
Dorsal aorta
Coeliac a.
Cranial mesenteric a.
Adrenal gland
Cranial superior renal a.
Suprarenal a.
Testis
Testicular aa.
Region of efferent ducts
Caudal superior renal a.
Epididymal region
External iliac a.
Lateral lumbar a.
Inferior renal a.
External ischiatic a.
Median sacral a.
Caudal mesenteric a.
Lateral sacral a.
Internal pudendal a.
Deferent duct
Muscular ramus of int. pud. a.
Atrophied cloacal bursa
Cloaca
Median coccygeal a.
Ejaculatory duct
Paracloacal glomus (Cavernous body)
Ventral lip
Vent
Dorsal lip
Cloaca

FIG. 1.14 — Ventral view of male urogenital system of the chicken. Main arteries in the thoracic; v.-vv., vein-veins. (from USDA). region are included. a.-aa., artery-arteries; int. pud., internal pudendal; L., lumbar; T.,

[38]

Inferior vena cava

Common vertebral v.
Rib T₄

Rib L₁

Efferent renal v.
Afferent renal v.
Renal portal valve
External iliac v.

Afferent renal v.
(s. renal portal v.)

External ischiatic a.

Efferent renal v.

Ischiatic v.

Caudal mesenteric a.

Hypogastric v.

Suprarenal vv.
Adrenal gland
Testicular vv.
Testis

External iliac a.

Dorsal aorta
Coccygeomesenteric v.

Medial sacral a.

Rectal ramus of
coccygeomesenteric v.
Internal pudendal a.

Median coccygeal a.

Common coccygeal v.

FIG. 1.15 — Ventral view of renal portal system in the male chicken. a., artery; L., lumbar; s., synonym; T., thoracic; v.—vv., vein—veins. (From USDA.)

[39]

found in some beta islets of nearly all chickens examined (Lucas, 1947). Well-illustrated accounts of the avian pancreas have been given by Clara (1921) and Nagelschmidt (1939).

Cloacal Bursa

The cloacal bursa is an ovoid, hollow organ that has a duct leading to the dorsal part of the proctodeum (Fig. 1.13). The shape is variable in different species of birds (Dominic, 1959–60) but in general varies from tubular to piriform. In the chicken, the inner surface is thrown into 11 to 13 primary plicae and 6 or 7 secondary plicae. The lining of the cavity is a simple columnar or pseudostratified epithelium derived from the endoderm. Below this is the *tunica propria* that contains numerous lymphoid follicles. Beyond the follicles are a muscularis layer and a connective tissue covering. Whether the lymphocytes of the *tunica propria* are derived solely from the mesenchyme or in part from the epithelial buds that form the medulla of the follicle has been a controversial question (Ackerman and Knouff, 1959, Ackerman, 1962). It is generally agreed that the epithelial buds produce the reticulum of the medulla and that the cortex is derived from mesenchyme (Boyden, 1922).

EXCRETORY SYSTEM

The excretory organs of the hatched bird are the metanephric kidneys, ureters, and the urodeal portion of the cloaca (Figs. 1.14, 1.15, and 1.16) and their function is supplemented by the nasal gland (p. 28). The kidneys are located caudal to the lungs and extend from the space between the last thoracic and the first lumbar ribs to approximately the level of the last vertebra of the synsacrum. The posterior end of each kidney partially fills the renal fossa, the remainder of the space being occupied by adipose tissue and the sacral diverticulum of the abdominal air sac. The avian kidney has a retroperitoneal location as in mammals but the membrane

covering it in birds is more complex because the dorsal wall of the abdominal air sac has fused with the peritoneum. The fusion extends from the ventral surface of the deferent duct to the middle of the lateral body wall. The remainder of the abdominal air sac is free and is separated from the peritoneum.

The kidney is divided into 3 lobes; the constriction between anterior and middle lobes on the ventral side of the organ is made by the external iliac (s. crural) vein and dorsally by the external iliac artery. The constriction between middle and posterior lobes is produced by the external ischiatic artery (Figs. 1.14 and 1.15).

The ureter emerges from the medial, ventral surface of the middle lobe, just anterior to the ischiatic artery. The duct within the kidney extends close and parallel to the medial margin (Fig. 1.14). It receives numerous branches throughout its length from all of the kidney lobes. The duct passes dorsal to the external iliac vein and ventral to the external ischiatic artery. The ureter runs along the medial edge of the deferent duct in the male and has an equivalent position in relation to the oviduct in the female (Fig. 1.16). Within the kidney parenchyma the ureter subdivides into collecting ducts. These in turn receive urinary fluids from the terminal end of each nephron by connecting tubules. The extrarenal ureter has a muscular wall and elimination of fluids and uric acid crystals is by peristaltic contractions toward the cloaca (Gibbs, 1929).

The nephron of reptiles and birds has glomeruli that are small in size and in birds contain but few capillary tufts. Marshall and Smith (1930) described a central cell mass which Pak Poy and Robertson (1957) in their electron microscopy analysis concluded were cells of the adventitia of the glomerular capillaries and functioned to support these capillaries. Marshall (1934) estimated the number of glomeruli in the kidneys of a fowl of 2,500 grams body weight to be about 814,000 with an average glomerular diameter of 75 microns. In a
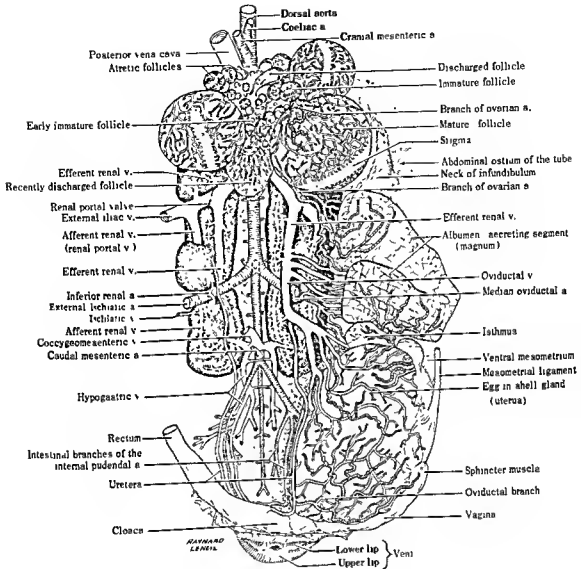
FIG. 1.16 — Ventral view of the female urogenital system of the chicken. a., artery; v., vein. (From USDA.)

2,400 gram rabbit, 414,000 glomeruli were estimated for both kidneys and the average diameter was 142 microns. Marshall and Smith (1930) regard the small glomerulus and central cell mass as part of a phylogenetic degeneration process in the avian kidney.

Immediately distal to the glomerulus is a short neck; this is not ciliated in birds and mammals as it is in lower vertebrates.

Following this is the proximal convoluted tubule which has the form of the letter N, as shown by Huber (1917), most of which lies in the cortical portion of the kidney but some of it may extend into the medullary area. A constricted tube lies between the proximal and convoluted tubules. This may be short as in reptiles or long and narrow as in Henle's loop of mammals. Marshall (1934) has given a

Female—Ovary

Normally in the female reproductive system of most birds, only the left ovary is functional; the right regresses during development but for the period soon after hatching it retains the potentiality of development into a testis that may contain sperm or into a functional ovary. Both right and left ovaries are commonly present and functional in hawks and owls. The left ovary is firmly attached by a short mesovarium near the mid-line lateral to the dorsal mesentery. The oviduct is suspended dorsally by the mesosalpinx; ventrally by the mesometrium; the latter is not attached to the body wall but its free border carries many muscular bundles and connective tissue ligaments (Fig. 1.16). The ovary has a cortex and a medulla; the former is composed of a germinal epithelium and an underlying stroma in which layer the follicles develop. The medulla bears the major vessels of the mesovarium.

The ovary, located at the anterior ends of the kidneys, is in the recently hatched chick a flat irregularly shaped gray body with a granular surface. The surface character is due to the presence of innumerable primary oocytes, of which only relatively few will reach maturity and be ovulated. The germinal epithelium lies upon a dense connective tissue layer, the *tunica albuginea*. Beneath this layer lies the stroma of the cortex. The ovary of the laying hen is a large body from the surface of which project follicles in all stages of yolk accumulation. The quiescent follicles in the cortex give to the surface a granular texture. The follicles during development become large and pendulous, the stalks of which carry their vascular supplies. Isolated interstitial cells are present in the stroma, the cytoplasm of which is granular.

During the embryonic development of the ovary a group of germinal epithelial cells invaginates and one of the group forms the ovum, the remainder arrange themselves around the young ovum and form a single layer of "nurse cells" that constitute the *membrana granulosa*. It is these cells in mammals that produce the *corona radiata* but they are not present in the avian egg at the time of ovulation. A double-layered vitelline membrane is produced between the yolk and the *membrana granulosa*.

The germinal disc at the surface of the growing ovum contains the egg nucleus surrounded by a small amount of cytoplasm that merges into the surrounding yolk spheres. The yolk is of two types, white and yellow. The white yolk at the center of the ovum extends to the area beneath the blastodisc and extends laterally as thin concentric spherical laminae separating the wider laminae of yellow yolk.

As the ovum increases in size the adjacent connective tissues of the stroma become organized into the follicular theca of which, in the older stages, a *theca interna* and *externa* may be distinguished. The tissues of the latter merge into the less compact connective tissue of the general stroma. In the internal theca as well as in the stroma are groups of luteal cells.

Bradley and Grahame (1960) have classified ovarian follicles into 5 phases:

1. Primary follicles.
2. Growing follicles.
3. Mature follicles.
4. Discharged follicles.
5. Atretic follicles.

During the process of growth, the theca becomes highly vascularized, particularly in regard to an extensive venous network. On the surface of the follicle opposite the stalk, a slit-shaped area, called the *stigma*, is not as well vascularized as the adjacent area (Nalbandov and James, 1949). The ovum leaves the follicle through a break in the stigma. This produces the "discharged follicle." The thecal cells proliferate. Later the whole structure de-

hypophysis is situated ventrally or rostro-ventrally to the neurohypophysis; it consists of a *pars distalis* and a *pars tuberalis*, and does not have a *pars intermedia*. Within the *pars distalis*, glandular cells are arranged in anastomosing cords and lamellae that are wrapped in a delicate double-layered membrane. A dense plexus of wide blood sinusoids surrounds and partly separates the cords. The several types of cells which make up the cords are distinguished and named according to their reactions to stains (Fayez and Rappay, 1961a and b; Ezrin, 1963). In many birds the *pars distalis* can be divided into cephalic and caudal regions on the basis of the distribution of acidophils (alpha cells) (Rahn and Painter, 1911; Payne, 1942). The boundary between these zones extends from the base of the *pars tuberalis* to the former site of attachment to the diencephalon. Other structures that have been found in the *pars distalis* at various degrees of frequency include pigment granules, colloid-filled cysts, hyperplastic epithelial tissue, and nodules of lymphoid tissue (Payne, 1942; Oboussier, 1948; Payne and Breneman, 1952).

The *pars tuberalis* is a thin layer of cells that forms a collar around the infundibulum and extends forward on the ventral surface of the diencephalon to the region of the optic chiasma. Its histology resembles that of the *pars distalis* except that most of the cells are chromophobes.

A sheath of fibrous connective tissue separates the anterior and the posterior lobes of the pituitary. The latter has two parts, the neural lobe (lobus nervosus or infundibular process) and the infundibulum (neural stalk). The neural lobe is usually a heart shaped or arrow-shaped body, pointed caudally or ventrocaudally, and situated above the caudal region of the *pars distalis*. The infundibulum has hitherto included the infundibular stem and the median eminence, but recent workers tend to consider the eminence as part of the hypothalamus A tubular projection of the diencephalic wall, the stem, connects the neural lobe to the *pars distalis*. Together with the *pars tuberalis* it is sometimes referred to as the hypophysial stalk. The neural lobe and the infundibular stem have a lumen which is continuous with the third ventricle of the brain; in many chickens it is so extensive that the neurohypophysis is entirely hollow (Oboussier, 1948). The median eminence *(eminentia mediana)* is the portion of the ventral or rostroventral wall of the diencephalon between the infundibular stem and the optic chiasma. Anatomically and functionally it may belong to the hypothalamus more than to the pituitary.

The lumen of the neurohypophysis is lined with a single layer of ependymal cells. Outside this is a thick fibrous layer of unmyelinated nerve fibers and several forms of pituicytes (modified glial cells). Beyond this is a palisade layer containing other types of pituicytes, neurosecretory axons, other elements, and masses of neurosecretory granules. This layer is thick in the median eminence but poorly developed in the infundibular stem (Payne, 1959; Kobayashi *et al.*, 1961; Oota and Kobayashi, 1963).

All arteries to the pituitary are branches of the internal carotids, arising from an intercarotid anastomosis. Blood from either the infundibular or the inferior hypophysial arteries enters the neural lobe by way of a superficial plexus and drains directly into the cavernous sinus (dural sinus) in the sella turcica below. A separate net, the primary plexus, on the surface of the median eminence, receives blood from either the infundibular or the superior hypophysial arteries. As in other vertebrates, hypophysio-portal vessels along the *pars tuberalis* carry the blood from the primary plexus to an extensive secondary plexus in the *pars distalis*. Efferent veins lead from here to the cavernous sinus, where blood from the pituitary meets that from the orbit and the forebrain. Blood leaves the sinus by veins which emerge from the skull through the canal for the carotid arteries, just anterior to the external audi-

tory meatus. These veins join the posterior cephalic veins, which in turn join the jugular vein (Neugebauer, 1845).

The pituitary gland is innervated by a hypophysial tract (hypothalamico-hypophysial fasciculus) which arises mostly from neurosecretory nuclei in the hypothalamus. The two component tracts pass along the hypophysial stalk and terminate, one in the median eminence and the other in the neural lobe (Drager, 1945; Green, 1951; Kobayashi *et al.*, 1961). A few fibers go on to the glandular cells of the *pars tuberalis* but none enter the *pars distalis*. The entire gland is said to be innervated by a plexus of the peripheral autonomic nervous system, and the *pars distalis* is further said to contain a very dense secreto-motor end-plexus (Metuzals, 1956).

Most of the pituitary hormones are secreted by the *pars distalis*. Production of adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) is regulated by neurohumors that are transported from the hypothalamus by the portal circulation. ACTH and the thyroid-stimulating hormone (TSH) were thought to come from acidophils (Höhn, 1961), but recent findings in mammals indicate types of basophils (beta-1 and beta-2 cells) as the origin (Ezrin, 1963). FSH and LH are produced by two other types of basophils (delta-1 and delta-2 cells) (Fayez and Rappay, 1961a, b; Ezrin, 1963). Prolactin and possibly a growth hormone are produced by two other types of acidophils. Intermedin, which is secreted by the *pars intermedia* of the adenohypophysis in other vertebrates, is formed in the *pars distalis* in birds.

The neurohypophysial hormones of birds are oxytocin and arginine vasotocin; the existence of vasopressin is currently uncertain (Munsick *et al.*, 1960). As in mammals, they are produced in the hypothalamic nuclei, transported in granules along the neurosecretory axons of the hypophysial tract, and stored in the neuro-

hypophysis (Farner and Oksche, 1962). It is no longer believed that additional secretions are produced in the neurohypophysis itself (cf. Payne, 1959).

Adrenal Glands

These glands are paired bodies situated beneath and medially to the anterior lobes of the kidneys, above the gonads, and behind the lungs (Fig. 1.14). They are commonly ovoid, curved, or triangular, but their shape is highly variable among species and individuals and may be polyhedral or flattened and irregular. The glands are cream, yellow, or orange in most species, but they may be white, grey, pink, or brown; color may depend on the lipid content. A gap of variable width usually separates the bodies though in rare cases they are united (Hartman and Albertin, 1951).

The following account of adrenal histology is based on the work of Müller (1929), Knouff and Hartman (1951), and Sinha *et al.* (1959). Each gland is enclosed in a double-layered fibrous capsule which also includes blood vessels, lymph vessels, nerve ganglia, and extrinsic sex ducts. The parenchyma is composed of interrenal (cortical) and chromaffin (medullary) tissues, but these are intermingled rather than zonated as in mammals. The two tissues vary widely in arrangement and relative amount among species of birds (Hartman *et al.*, 1917). They are present in approximately equal volume in White Leghorn chickens, but even here there are variations between sexes and among lines (Oakberg, 1951).

The interrenal cells form cords that wind inward from the periphery of the gland. Each strand is built of radially arranged spherical or cylindrical cells, with a foamy cytoplasm and a deeply staining nucleus near the axis of the cord. Mitochondria, several kinds of lipid droplets (Fayez and Rappay, 1961a, b), and other inclusions are distributed in the cytoplasm. The amounts of lipids and mitochondria are inversely related to each other, and de-

pend on the functional state of the gland (Morita et al., 1961).

The chromaffin tissue takes the form of strands or masses that are sometimes connected by bridges. These cells are irregularly arranged except at the periphery of the gland. They are large and polyhedral, with intensely basophilic granular cytoplasm and vacuolated nuclei. Patches of chromaffin tissue may occur in the capsule of the gland and, as paraganglia, elsewhere in the body. The interrenal and chromaffin tissues are separated by a delicate capillary plexus and a few fibroblasts. Blood from the internal spermatic arteries reaches each gland through a suprarenal artery and branches of the cranial superior renal artery. These vessels send arterioles into the pericapsular sheath, to the plexus, and to central venous sinuses. Large capsular veins collect the blood from the central and the peripheral sinuses, and return it by way of short suprarenal veins to the inferior vena cava.

Several large sympathetic nerves supply ganglia and a rich plexus in the capsule, from whence fibers penetrate the parenchyma to the chromaffin masses. Most of the fibers are myelinated.

The avian adrenal hormones are similar to those of mammals but they are not as well known. The interrenal tissue secretes three main kinds of hormones: mineralocorticoids (e.g., aldosterone), gonadoids (e.g., estrogens and androgens), and glucocorticoids (e.g., cortisone, hydrocortisone, and corticosterone) (de Roos, 1961). The mineralocorticoids regulate the resorption of sodium and potassium from the blood in the kidneys. The gonadoids seem to play a role in the endocrine and spermatogenetic activity of the testes. They may have a masculinizing effect on chickens under abnormal conditions, but such a function is not yet known in normal birds. The glucocorticoids help to control blood sugar and the deposition of glycogen in the liver. The production and action of the interrenal secretions are regulated by the adrenocorticotropic hormone of the pitui-

tary, but the size of the adrenals is not controlled by this hormone as rigidly in birds as in mammals (Zarrow et al., 1962).

The chromaffin tissue secretes adrenalin and noradrenalin in a proportion that varies widely among birds. Noradrenalin accounts for 50 to 60 per cent of the total secretion in the domestic pigeon and 70 to 80 per cent in the fowl (Ghosh, 1962). Both hormones take effect rapidly though briefly, mobilizing body functions to meet stress.

Thyroid Glands

These are a pair of dark red, ovoid bodies on the ventro-lateral surfaces of the neck, just anterior to the first cervical ribs (Fig. 1.17). They lie posterior or deep to the last lobe of the thymus and anterior to the parathyroid glands. The avian thyroids, unlike the mammalian, do not touch the trachea and are not joined by an isthmus. Their size is highly variable, depending on many factors (Ringer, in press).

Each gland is a mass of round to oval follicles within a capsule of fibrous connective tissue. The follicles are lined with a single layer of endodermal epithelial cells on a poorly defined basement membrane. The cells vary in height from columnar to squamous, depending on age, location, and state of activity (Payne, 1957; Yamamoto, 1960). Their nuclei are round or oval, and the cytoplasm stains faintly basophilic and contains many tiny vacuoles. Within each follicle is a homogeneous colloid containing thyroglobulin (protein-bound thyroxine), its quantity varying with the activity of the gland. Cells that appear identical to those of the parathyroid gland occur between the follicles usually in thin septa but occasionally in masses.

Blood from the common carotid arteries reaches the glands by way of the ascending esophageal arteries and their branches, the cranial and caudal thyroid arteries. These supply dense networks of capillaries around the follicles. A sphincter apparatus of endothelium and circular smooth

muscle fibers is sometimes present at the bifurcations of the arteries (Yamamoto, 1960). Owing to the thinness of the interstitial tissue and the basement membrane, the endothelium of the capillaries is almost in direct contact with the follicular epithelium (Ringer, in press). Thyroid veins return the blood to the jugular veins. The innervation of the avian thyroid is not known, but it may come from the cervical sympathetic ganglion as in mammals.

The thyroid gland releases the hormones thyroxine and triiodothyronine that tend to increase oxidative metabolism (Wentworth and Mellen, 1961). They are necessary for normal growth and development, especially of reproductive characters and functioning (Blivaiss, 1947). The enormous amount of research on the functioning of the thyroid has been recently reviewed by Ringer (in press).

Parathyroid Glands

A pair of parathyroid glands is present on each side of the base of the neck, near the posterior end of the thyroids (Fig. 1.17). In pigeons, the lobes of each pair are separated from each other and from the thyroid, whereas in fowl they are sometimes fused together and even attached to the thyroid. If separated from that gland, the parathyroids lie ventral to the jugular vein and dorsal to the common carotid artery. They are spherical to elongate, yellowish-pink or yellowish-white bodies, usually much smaller than the thyroid but sometimes almost half its size (Forsyth, 1908). The anterior parathyroid is usually the larger, and the glands are slightly larger in females than in males.

Each body is enclosed in a capsule of thin connective tissue which is continuous with the tunica externa of the carotid artery. The glands are masses of anastomosing cords supported by delicate strands of connective tissue which are connected to the capsule. Such structure resembles that of the thyroid except that the cords are thicker and there are no vesicles. The cords are composed of closely packed,

columnar epithelial cells, surrounded by a basement membrane (Nonidez and Goodale, 1927). Most numerous are cells with an acidophilic cytoplasm and an intensely staining nucleus (Romanoff, 1960). Oxyphil cells, as found in mammals, are absent. Masses of accessory parathyroid tissue with histologic structure similar to the glands, may be present in the caudal lobe of the thymus, under the capsule of the thyroid, or in the ultimobranchial bodies (Thompson, 1910; Nonidez and Goodale, 1927).

Blood reaches the glands by a short parathyroid artery close behind the caudal thyroid artery, either directly from the common carotid artery or a branch of it: the ascending esophageal artery. The connective tissue stroma supports a rich plexus of capillaries, some of them sinusoidal, with endothelial walls that are nearly in contact with the epithelial cords. Venous blood returns directly to the jugular veins.

The parathyroid hormone (parathormone) plays important roles in regulating the level of calcium in the blood and the renal excretion of phosphorus.

Ultimobranchial (Postbranchial) Bodies

These structures are closely associated with the thymus and the parathyroid glands but they have no known endocrine function. They are situated one on each side of the neck, posterior to the parathyroids, medial to the jugular veins, and anterodorsal to the subclavian artery (Fig. 1.17). The bodies are small, subspherical, or polyhedral, and pinkish (fowl) or yellowish white (pigeon); in some individuals they may be irregular masses or even absent.

They are composed chiefly of compact cords of polyhedral epithelial cells (similar to parathyroid tissue) and spherical or tubular cysts. The latter are lined with squamous or cuboidal epithelium that may be ciliated and are filled with cellular debris or colloid. One or more islets of parathyroid tissue, ensheathed in collagenic tissue, usually occur in the interior of each ultimobranchial body. Nodules of

Botezat, E: 1910. Morphologie, Physiologie und phylogenetische Bedeutung der Geschmacks-organe der Vögel Anat. Anz. 36.428.

Boulton, R.: 1927. Ptilosis of the house wren (*Troglodytes aedon aedon*). Auk 41:387.

Boyden, E. A.: 1922. The development of the cloaca in birds, with special reference to the origin of the bursa of Fabricus, the formation of the urodaeal sinus, and the regular occurrence of a cloacal fenestra Am. Jour. Anat 30 163.

Bradley, O C., and Grahame, T.: 1960. The Structure of the Fowl Oliver and Boyd, Edinburgh and London 4th ed. xii + 143 pp

Buchholtz, V.: 1939-60. Beitrag zur makroskopischen Anatomie des Armgeflechtes und der Beck-ennerven beim Haushuhn (*Gallus domesticus*). Wiss Zeitschr. Humboldt-Univ. Berlin, Math.-Nat. R. 9.565.

Burggraaf, P. D · 1954. On the correlation between the skull structure and the muscles in the male *Phasianus colchicus* L. V. The attachment of the *musculus mylohyoideus* and of the *musculus genuohyoideus* Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C, 57·673

——, and Fuchs, A.: 1954. On the correlation between the skull structure and the muscles in the male *Phasianus colchicus* L. I. General introduction. Koninkl. Nederl. Akad. Van Weten-schappen — Amsterdam. Proc., Ser. C. 57:286

——, and Fuchs, A.: 1955. On the correlation between the skull structure and the muscles in the male *Phasianus colchicus* L. VII. General considerations. Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C 58 98

Burrows, W. H., and Quinn, J. P. 1937. Collection of spermatozoa from the domestic fowl and turkey Poultry Sci. 16.19

Butler, G. A.: 1889 On the subdivisions of the body-cavity in lizards, crocodiles and birds Proc. Zool. Soc London (1889):452.

Calhoun, M L 1954 Microscopic Anatomy of the Digestive System of the Chicken. Iowa State Univ Press, Ames, Iowa ix + 110 pp

Carpenter, F W: 1906 The development of the oculomotor nerve, the ciliary ganglion, and the abducent nerve in the chick. Bull Mus. Comp. Zool. Harvard Coll. 48:141.

Champy, C., and Demay, M.: 1939 Structure et homologie des papilles charnues des régions denudées de la tête des gallinacés Bui. Soc. Zool. France 55·410.

——, and Kritch, N: 1925. Le tissu muco-élastique de la crête du coq, réactif de l'hormone sexuelle (avec démonstration) Comp. rend. Soc. d. Biol. 92.633

——, and Kritch, N · 1926. Étude histologique de la crête des gallinacés et de ses variations sous l'influence des facteurs sexuels. Arch Morph. gen. exp. Paris 25.1.

Chowdhary, D. S. 1953 Carotid Body and "Carotid Sinus" of the Fowl (*Gallus domesticus*). Thesis for Ph.D.— Univ. of Edinburgh.

Clara, M · 1924. Das Pankreas der Vögel. Anat. Anz. 57:257.

—— 1927. Beitrage zur Kenntnis des Vogeldarmes VIII. und letzter Teil. Das Problem des Rumpfdarmschleimhautreliefs. Zeitschr. f. mikrosk. anat. Forsch. 9.1.

Cobb, S 1960. Observations on the comparative anatomy of the avian brain. Persp in Biol. and Med. 3:383

Cohen, H., and Davies, S: 1937 The development of the cerebrospinal fluid spaces and choroid plexuses in the chick Jour Anat 72.25.

Compton, L. V.: 1938 The pterylosis of the Falconiformes with special attention to the taxo-nomic position of the osprey. Univ. Cal. Publ. Zool. 42·173.

Cords, E.: 1904. Beiträge zur Lehre von Kopfnervensystem der Vögel. Anat Hefte 26:49.

Coulombre, A. J., Coulombre, J. L., and Mehta, H.: 1962 The skeleton of the eye. 1. Con-junctival papillae and scleral ossicles. Devel. Biol. 5 382

Cover, M S · 1953a. The gross and microscopic anatomy of the respiratory system of the turkey. I The nasal cavity and infraorbital sinus Am. Jour. Vet. Res. 14:113.

—— 1953b Gross and macroscopic anatomy of the respiratory system of the turkey, II. The larynx, trachea, syrinx, bronchi, and lungs. Am. Jour. Vet. Res. 14:230.

—— 1953c. Gross and microscope anatomy of the respiratory system of the turkey. III. The air sacs Am. Jour. Vet. Res. 14:239

Curtis, E. L., and Miller, R. C: 1938 The sclerotic ring in North American birds. Auk 55:225.

Davids, J. A. G.: 1952. Étude sur les attaches au crâne des muscles de la tête et du cou chez *Anas platyrhyncha platyrhyncha*. Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C. 55:81, 525.

Den Boer, P J.: 1953. On the correlation between the cervical muscles and the structure of the skull in *Phasianus colchicus* L and *Perdix perdix* L Koninkl. Nederl. Akad. Van Weten-schappen — Amsterdam. Proc., Ser. C. 56:335, 455.

De Gennaro, L. D · 1959. Differentiation of the glycogen body of the chick. Anat. Rec. 133·161.

Dennington, E. M., and Lucas, A. M. · 1960 Influence of heat treatment on the number of ectopic lymphoid foci in chickens. Am. Jour. Vet. Res. 21:734.

Dickson, A. D., and Millen, J W.: 1957. The meningeal relationships of the glycogen body in the chick. Jour. Anat. 91·47.

Dominic, C. J.: 1959-60. The structure of the bursa of Fabricius in some Indian birds. Jour. Sci. Res. Banaras Hindu Univ. 10:217.

Doyle, W. L.: 1960. The principal cells of the salt-gland of marine birds. Exper. Cell Res. 21:386.

Drager, G. A.: 1945. The innervation of the avian hypophysis. Endocrinology 36:124.

Dransfield, J. W.: 1944. The lymphatic system of the domestic fowl. Thesis, Univ. of Liverpool.

———: 1945. The lymphatic system of the domestic fowl. Vet. Jour. London 101:171.

Duncan, C. J.: 1960. The sense of taste in birds. Ann. Appl. Biol. 48:409.

Eglitis, I., and Knouff, R A.: 1962. An histological and histochemical analysis of the inner lining and glandular epithelium of the chicken gizzard. Am. Jour. Anat. 111:49.

Eisner, E.: 1960. The relationship of hormones to the reproductive behaviour of birds, referring especially to parental behaviour; a review. Animal Behaviour 8:155.

Ezrin, C.: 1963. The pituitary gland. Clinical Symposia 15:71.

Fahrenholz, C.: 1937. Drüsen der Mundhöhle. In Bolk, L., Göppert, E., Kallius, E., and Lubosch, W. Handbuch der vergleichenden Anatomie der Wirbeltiere. Urban and Schwarzenberg, Berlin and Vienna. 3:115.

Fänge, R., Schmidt-Nielsen, K., and Osaki, H.: 1958. The salt glands of the Herring Gull. Biol. Bul. 115:162.

Farner, D. S.: 1958. Photoperiodism in animals with special reference to avian testicular cycles. In Photobiology. Oregon State Coll. Biol. Colloquium 19:17.

———, and Oksche, A.: 1962. Neurosecretion in birds. Gen. and Comp. Endocrinology 2:113.

Fayez, M. A., and Rappay, G.: 1961a. Notes on the histochemistry of the adenohypophysis and adrenal gland of the female domestic pigeon (Columba domestica). Acta Biol. Acad. Sci. Hungaricae 12:127.

———, and Rappay, G.: 1961b. Notes on the histochemistry of the adenohypophysis and adrenal gland of the domestic fowl (Gallus domesticus). Acta Biol. Acad. Sci. Hungaricae 12:133.

Fisher, H. I.: 1940 The occurrence of vestigial claws on the wings of birds. Am. Midl. Nat. 23:234.

———: 1946. Adaptations and comparative anatomy of the locomotor apparatus of New World Vultures. Am Midl Nat. 35:545.

———, and Goodman, D. C.: 1955. The Myology of the Whooping Crane, Grus americana. Ill. Biol Monogr. 24 (2). Univ. of Illinois Press, Urbana, Ill. vi + 127 pp.

Fleming, R. E.: 1926. The origin of the vertebral and external carotid arteries in birds Anat. Rec. 33:183.

Fleury, S.: 1902. Recherches sur la structure des ganglions lymphatiques de l'oie. Arch. Anat. Micr. 5:38.

Forbes, W. A.: 1877. On the bursa Fabricii in birds. Proc. Zool. Soc. London (1877):304.

Forsyth, D.: 1908. The comparative anatomy, gross and minute, of the thyroid and parathyroid glands in mammals and birds. Jour. Anat. and Physiol. 42:141, 302.

Freedman, S. L., and Sturkie, P. D.: 1963. Blood vessels of the chicken's uterus (shell gland). Am. Jour. Anat. 113:1.

Fuchs, A.: 1954. On the correlation between the skull structure and the muscles in the male Phasianus colchicus L. IIIA. The attachment of the musculus adductor mandibulae posterior and the musculus adductor mandibulae internus. Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C. 57:454.

———: 1955. On the correlation between the skull structure and the muscles in the male Phasianus colchicus L. VI Some remarks on a number of ligaments and other connective tissue connections. Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C. 58:114.

Fürbringer, M.: 1888. Untersuchungen zur Morphologie und Systematik der Vögel, zugleich ein Beitrag zur Anatomie der Stutz — und Bewegungsorgane. van Holkema, Amsterdam. 2 vols. xlix + 1751 pp.

Further, H.: 1913. Beiträge zur Kenntnis der Vogellymphknoten. Jena. Zeitschr. f. Naturw. 50:559

Gadow, H.: 1879. Versuch einer vergleichenden Anatomie des Verdauungssystems der Vögel. Jena. Zeitschr. f. Naturw. 13:92, 339.

——— 1888. Remarks on the cloaca and on the copulatory organs of the amniota. Roy. Soc. London, Phil. Trans., Ser. B. 178.5.

——— 1889. On the taxonomic value of the intestinal convolutions in birds. Proc. Zool. Soc London (1889):303.

———, and Selenka, E.: 1891. Vögel. In Bronn, H. G. Klassen und Ordnungen des Thierreichs. C. F. Winter, Leipzig. 6, Abt. 4:1.

Ganfini, C.: 1930. Sulla origine degli elementi che formano la bolla gelatinosa del midollo lombo-sacrale degli uccelli. Riv. Sper. Freniat. 54:494.

George, J. C., and Naik, R. M.: 1957. Studies on the structure and physiology of the flight muscles of birds. I. The variations in the structure of the pectoralis major muscle of a few representative types and their significance in the respective modes of flight. Jour. Anim. Morph. Physiol. 4:23.

George, J. C., and Naik, R. M.: 1958. Relative distribution of the mitochondria in the two types of fibers in the pectoralis major muscle of the pigeon. Nature 181:782.

———, and Naik, R. M.: 1959. Studies on the structure and physiology of the flight muscles of birds. 4. Observations on the fiber architecture of the pectoralis major muscle of the pigeon. Biol. Bull. 116:239.

———, and Talesara, C. L.: 1962. Histochemical demonstration of certain oxidizing enzymes in the *pectoralis major* muscle of the rosy pastor (*Pastor roseus*), goose (*Anser albifrons*) and fowl (*Gallus domesticus*). Jour. Anat. Morph. Physiol 9.59

Gerber, A.: 1939. Die embryonale und postembryonale Pterylose der Alectoromorphae. Rev. suisse de Zool 46:161.

Ghosh, A.: 1962 A comparative study of the histochemistry of the avian adrenals. In Third International Symposium on Comparative Endocrinology, Oiso, Japan, June 1961, Gen. and Comp Endocrinology, Suppl. 1:75.

Gibbs, O. S.: 1929 The function of the fowl's ureters. Am. Jour. Physiol. 87:594.

Glenny, F. H.: 1955. Modifications of pattern in the aortic arch system of birds and their phylogenetic significance. Proc. U.S. Nat. Mus. 104:525.

Grahame, T.: 1938. The sympathetic and parasympathetic nervous systems of the fowl. Notes on a thesis presented for the degree of doctor of philosophy. Brit. Vet. Jour. 109:481.

Gray, J. C.: 1937. The anatomy of the male genital ducts in the fowl. Jour. Morph. 60:393.

Green, J. D.: 1951 The comparative anatomy of the hypophysis with special reference to its blood supply and innervation. Am. Jour. Anat. 88:225.

Gregory, W. K.: 1920. Studies in comparative myology and osteology: No. IV. A review of the evolution of the lacrymal bone of vertebrates with special reference to that of mammals. Bul. Am. Mus. Nat. Hist. 42:95.

Greschik, J.: 1916. Zur Histologie der Vogelhaut. Die Haut des Kernbeissers und Haussperlings. Aquila 22 89.

——— 1917. Geschmacksknospen auf der Zunge des Amazonenpapagels Anat. Anz. 50:257.

Haefelinger, H R 1958. Beitrage zur vergleichenden Ontogenese des Vorderkiemers bei Vögeln.

Helbing & Lichtenhahn, Basel, 99 pp.

Hamilton, H. L.: 1952. Lillie's Development of the Chick. Henry Holt & Co., N.Y. xv + 624 pp.

Hammond, W S., and Yntema, C. L.: 1947. Depletions in the thoracolumbar sympathetic system following removal of neural crest in the chick. Jour. Comp. Neur. 86:237.

———and Yntema, C. L.: 1958 Origin of ciliary ganglia in the chick. Jour. Comp. Neur. 110.367

Hansen Pruss, O. C.: 1923. Meninges of birds, with a consideration of the sinus rhomboidalis. Jour. Comp. Neur. 36:193.

Hartman, F. A., and Albertin, R. H.: 1951. A preliminary study of the avian adrenal. Auk 68 202.

———, Knouff, R. A., McNutt, A W., and Carver, J. E.: 1947. Chromaffin patterns in bird adrenals. Anat Rec 97 211

Heidrich, P. K.: 1908. Die Mund- und Schlundkopfhöhle der Vogel und ihre Drüsen. Morph. Jahrb. 37.10.

Helm, F.: 1884 Über die Hautmuskeln der Vögel, ihre Beziehungen zu den Federfluren und ihre Functionen. Jour. Ornith., Ser. 4, 12:321.

Henn, R. A., Booth, N. H., and Johnson, R. M.: 1960. Thermoregulatory effects of abdominal air sacs on spermatogenesis in domestic fowl. Am Jour. Physiol. 198:1343

Höhn, E. O.: 1959. Action of certain hormones on the thymus of the domestic hen. Jour. Endocrinology 19 282.

——— 1961. Endocrine glands, thymus and pineal body. In Marshall, A. J. Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:87.

Holmes, A. 1935. The pattern and symmetry of adult plumage units in relation to the order and locus of origin of the embryonic feather papillae. Am. Jour. Anat. 56:513.

Holmgren, N.: 1955. Studies on the phylogeny of birds. Acta Zool. 36:243

Howard, H.: 1929. The avifauna of Emeryville shell mound. Univ. Calif. Publ. Zool 32:301.

Howell, A. B.: 1937. Morphogenesis of the shoulder architecture: Aves. Auk 54:363.

——— 1941. The femoral trochanters. Field Mus. Nat. Hist., Zool. Ser. 27(Publ. No. 511):279.

Hsieh, T. M.: 1951. The sympathetic and parasympathetic nervous systems of the fowl. Dept Anat, Royal (Dick) Sch. Vet. Stud., Edinburgh Univ. 192 pp.

Huber, G. C.: 1917. On the morphology of the renal tubules of vertebrates. Anat. Rec. 13.305.

——— 1936. Nerve roots and nuclear groups in the spinal cord of the pigeon. Jour. Comp. Neur 65:43

———, and Crosby, E.: 1929. The nuclei and fiber paths of the avian diencephalon, with consideration of telencephalic and certain mesencephalic centers and connections. Jour. Comp. Neur. 48:1.

Hudson, G. E.: 1937. Studies on the muscles of the pelvic appendage in birds. Am. Midl. Nat. 18:1.

————, and Lanzillotti, P. J.: 1955. Gross anatomy of the wing muscles in the family Corvidae. Am. Midl. Nat. 53:1.

————, and Lanzillotti, P. J.: 1964. Muscles of the pectoral limb in galliform birds. Am. Midl. Nat. 71:1.

————, Lanzillotti, P. J., and Edwards, G. D.: 1959. Muscles of the pelvic limb in galliform birds. Am. Midl. Nat. 61:1.

Hughes, A. F. W.: 1934–35. On the development of the blood vessels in the head of the chick. Phil. Trans. Roy. Soc., London, Ser. B. 224:75.

Humphrey, P. S., and Clark, G. A., Jr.: 1961. Pterylosis of the mallard duck. Condor 63:365.

International Anatomical Nomenclature Committee (IANC): 1956. Nomina Anatomica Williams & Wilkins, Baltimore. 51 pp.

Johnson, O. W.: 1961. Reproductive cycle of the mallard duck. Condor 63:351.

Jollie, M.: 1957. The head skeleton of the chicken and remarks on the anatomy of this region in other birds. Jour. Morph. 100:389.

Jolly, J.: 1910. Recherches sur les ganglions lymphatiques des oiseaux. Arch. d'Anat. micr. 11:179.

Josifoff, J. M.: 1930. Das Lymphgefässystem der Huhner und Tauben. Anat. Anz. 69:213.

Juillet, A.: 1912. Recherches anatomiques, embryologiques, histologiques et comparatives sur le poumon des oiseaux. Arch. Zool. Exp. Gén. (5th ser.) 9:207.

Junghern, E.: 1945. Certain nuclear groups of the avian mesencephalon. Jour. Comp. Neur. 82:55.

Kamar, G. A. R.: 1960. Developments of the endocrine glands in male Fayomi fowls. Acta Anat. 40:273.

Kanesada, A.: 1956. A phylogenetical survey of hemocytopoietic tissues in submammalian vertebrates, with special reference to the differentiation of the hemocyte and lymphoid tissue. Bul. Yamaguchi Med. Sch. 4:1.

Kern, A.: 1926. Das Vogelherz. Untersuchungen an Gallus domesticus Briss. Morph. Jahrb. 56:264.

Kihara, T., and Naito, E.: 1933. Über den Einlagerungs— und Verbreitungsmodus des lymphatischen Gewebes im Lymphgefässystem der Ente. Folia Anat. Jap. 11:405.

King, A. S.: 1956. The structure and function of the respiratory pathways of Gallus domesticus. Vet. Rec. 68:544.

————: 1957. The aerated bones of Gallus domesticus. Acta Anat. 31:220.

Kitoh, J.: 1962. Comparative and topographical anatomy of the fowl. XII. Observation on the arteries with their anastomoses in and around the brain in the fowl. Jap. Jour. Vet. Sci. 24:141.

Knopfli, W.: 1918. Beiträge zur Morphologie und Entwicklungsgeschichte des Brustschulterskelettes bei den Vögeln. Jena. Zeitschr. f. Naturw. 55:1.

Knouff, R. A., and Hartman, F. A.: 1951. A microscopic study of the adrenal of the brown pelican. Anat. Rec. 109:161.

Kobayashi, H., Bern, H. S., Nishioka, R. S., and Hyodo, Y.: 1961. The hypothalamo-hypophysial neurosecretory system of the parakeet, Melopsittacus undulatum. Gen. and Comp. Endocrinology 1:545.

Komarek, V.: 1958. Die Federfluren beim Haushuhn. 1. Teil. Bildung der Federfluren bei Frucht der weissen Leghorn-Henne. Acta Univ. Agri. et Silvi. Brno., Ser. B., Publ. Vet. Fac. 6:225.

Kondo, M.: 1937. Die lymphatische Gebilde im Lymphgefässystem der verschiedenen Vogelarten. Fol. Anat. Jap. 15:329.

Krabbe, K. H.: 1955. Development of the pineal organ and a rudimentary parietal eye in some birds. Jour. Comp. Neur. 103:139.

Lake, P. E.: 1957. The male reproductive tract of the fowl. Jour. Anat. 91:116.

Lakjer, T.: 1926. Studien über die Trigeminus-versorgte Kaumuskulatur der Sauropsiden. Ed. Luther, A., and Wesenberg-Lund, C. C. A. Reitzel, Copenhagen. 155 pp.

Lange, B.: 1928. Die Brutflecke der Vögel und die für sie wichtigen Hauteigentümlichkeiten. Morph. Jahrb 59.601.

————: 1931. Integument der Sauropsiden. In Bolk, L., Göppert, E., Kallius, E., and Lubosch, W. Handbuch der vergleichenden Anatomie der Wirbeltiere. Urban and Schwarzenberg, Berlin and Vienna. 1:375.

Langley, J. N.: 1904. On the sympathetic system of birds, and on the muscles which move the feathers. Jour. Physiol. 30.221.

Larsell, O.: 1918. The development and subdivisions of the cerebellum of birds. Jour. Comp. Neur. 89:123.

————, and Whitlock, D C.: 1952. Further observations on the cerebellum of birds. Jour. Comp. Neur. 97:515.

Latimer, H. B., and Rosenbaum, J. A.: 1926. A quantitative study of the anatomy of the turkey hen. Anat. Rec. 34:15

Lauth, E. A.: 1824. Mémoire sur les vaisseaux lymphatiques des oiseaux, et sur la manière de les préparer. Ann. Sci. Nat, Paris, 3.331.

Lebedinsky, N.: 1913. Beiträge zur Morphologie und Entwicklungsgeschichte des Vogelbeckens. Jena Zeitschr. f. Naturw. 50:1.

Leeuwrich, W.: 1931. Der Skleralring der Vögel. Jena. Zeitschr. f. Naturw. 65:513.

Liebels, R. A., and Eastlick, H. L.: 1951. The organ like nature of the subcutaneous fat bodies in the chicken. Poultry Sci. 33.169

Lindenmaier, P., and Kare, M.: 1959. The taste end organs of the chicken. Poultry Sci. 38:515

Locatelli, P.: 1927. Sur la structure du nerf olfactif. Arch. Ital. Biol. 77:208.

Lory, W. A., and Lazell, O.: 1916. Embryology of the bird's lung. Based on observations of the domestic fowl. Part 1. Am. Jour. Anat. 19-117. Part II. Am. Jour. Anat. 20.1.

Lucas, A. M.: 1917. Intranuclear inclusions in the islands of Langerhans of chickens. Am. Jour. Path. 23.1003

——: 1951. Lymphoid tissue and its relation to so-called normal lymphoid foci and to lymphomatous VI. A study of the lymphoid areas in the pancreas of doves and pigeons. Poultry Sci. 30.116

——, and Denington, E. M.: 1956. Morphology of the chicken liver. Poultry Sci. 35:793.

——, and Denington, E. M.: 1961. A brief report on anatomy, histology, and reactivity of air sacs in the fowl. Avian Dis. 5.460

——, Denington, E. M., Cotral, G. E., and Busmeater, B. R.: 1951. Production of so-called normal lymphoid foci following inoculation with lymphoid tumor filtrate. I. Pancreas II. Liver and spleen. Poultry Sci. 33.562

——, and Jamroz, C.: 1961. Atlas of Avian Hematology. Agriculture Monograph 25. U.S. Gov. Print Off (1 d. 271 pp.

Malewitz, T. D., and Calhoun, M. L.: 1938. The gross and microscopic anatomy of the digestive tract, spleen, kidney, lungs and heart of the turkey. Poultry Sci. 37:388.

Malinovsky, L.: 1962. Contribution to the anatomy of the vegetative nervous system in the neck and thorax of the domestic pigeon. Acta Anat. 50.326.

——: 1963 The nerve supply of the stomach in the domestic pigeon (Columba domestica). Morfologie 11-16.

Marshall, E. K., Jr.: 1931. The comparative physiology of the kidney in relation to the theories of renal secretion. Physiol. Rev. 14:133.

——, and Smith, H. W.: 1930. The glomerular development of the vertebrate kidney in relation to habitat. Biol. Bull. 59:135.

Metuzala, J.: 1955 The innervation of the adenohypophysis in the duck. Jour. Endocrinology 13.487

Müller, W. D.: 1924. Variations in the structure of the aftershaft. Am. Mus. Nat. Hist, Nov. #140.7pp

Mitchell, P. C.: 1901 On the intestinal tract of birds; with remarks on the valuation and nomenclature of zoological characters. Trans. Linn. Soc. London, Ser. 2 (Zool.) 8 173.

Mivart, G.: 1895. The skeleton of Lorius flavopalliatus compared with that of Psittacus erithacus. Part I. Proc. Zool. Soc. London (1895)-312-37.

Montagna, W.: 1945. A re-investigation of the development of the wing of the fowl. Jour. Morph. 76 87.

Moore, C. A., and Elliott, R. E.: 1946. Numerical and regional distribution of taste buds on the tongue of the fowl. Jour. Comp. Neur. 81:119.

Morita, S., Daigo, M., and Ogami, E.: 1961. Histological and histochemical studies of the adrenal cortex of domestic fowls. I. General histological studies of adrenal cortex and its lipids Jap. Jour. Vet. Sci. 23:325.

Moser, E.: 1906. Die Haut des Vogels. In Ellenberger, W. Handbuch der vergl. mikr. Anat. der Haustiere. 1:192

Mudge, G. P.: 1902 On the morphology of the tongue of parrots, with a classification of the order, based upon the structure of the tongue. Trans. Zool. Soc. London. 16.211.

Müller, B.: 1908. The air-sacs of the pigeon. Smithson. Misc. Coll 50:365.

Müller, J.: 1929 Die Nebennieren von Gallus domesticus und Columba livia domestica. Zeitschr. wiss.-anat. Forsch 17-503

Müller, S.: 1922. Zur Morphologie des Oberflächenreliefs der Rumpfdarmschleimhaut bei den Vögeln. Jena Zeitschr. Nat. 58 533.

Munsick, R. A., Sawyer, W. H., and Van Dyke, H. B.: 1960. Avian neurohypophysial hormones: pharmacological properties and tentative identification. Endocrinology 66 860.

Myers, J. A.: 1917 Studies on the syrinx of Gallus domesticus. Jour. Morph. 29.165.

Nagelschmitt, L.: 1939 Untersuchungen über die Langerhansschen Inseln der Bauchspeicheldruse bei den Vögeln. Jahrb. morph. u. mikr. Anat. (Abt. 2) 45:200.

Nalbandov, A. V., and James, M F.: 1949. The blood-vascular system of the chicken ovary. Am. Jour. Anat. 85 347.

Nelson, N.: 1942. The sclerotic plates of the White Leghorn chicken. Anat. Rec. 84:295.

Neugebauer, L. A.: 1845. Systema venosum avium. Verb. der kaiser. Leopold-carol. Akad. Naturf (Nova Acta Leopoldina) 21-517.

Nielsen, E. H.: 1963. The development of tarsus in *Gallus domesticus*. Acta Vet. Scand. 4:13.

Nishiyama, H.: 1954. Studies on the reproductive physiology of the cock. V. The influence of androgen on the accessory organs of the phallus. Proc. Tenth World's Poultry Cong. Sec. A:88.

————: 1955. Studies on the accessory reproductive organs in the cock. Jour. Fac. Agri. Kyûshû Univ. 10:377.

Nishida, T.: 1960. Comparative and topographical anatomy of the fowl. II. On the blood vascular system of the thoracic limb in the fowl. Part I. The artery. Jap. Jour. Vet. Sci. 22:223.

————: 1963. Comparative and topographical anatomy of the fowl. X. The blood vascular system of the hind-limb in the fowl. Part I. The artery. Jap. Jour. Vet. Sci. 25:93.

Nitzsch, C. L.: 1867. Pterylography. Ed. by Burmeister, H. (1840). Trans. by Dallas, W. S. and ed. by Sclater, P. L. (1867). Ray Soc. London (1867) xi + 178 pp.

Nonidez, J. F.: 1935. The presence of depressor nerves in the aorta and carotid of birds. Anat. Rec. 62:47.

————, and Goodale, H. D.: 1927. Histological studies on the endocrines of chickens deprived of ultraviolet light. Am. Jour. Anat. 38:319.

Oakberg, E. F.: 1950. Distribution and amount of lymphoid tissue in some of the splanchnic nerves of chickens in relation to age, sex and individual constitution. Poultry Sci. 29:420.

————: 1951. Genetic differences in quantitative histology of the adrenal, organ weights, and interorgan correlations in White Leghorn chickens. Growth 15:57.

Oboussier, H.: 1948. Über die Grössenbeziehungen der Hypophyse und ihrer Teile bei Säugetieren und Vögeln. Arch. f. Entwicklungsm. Organismen 143:18.

Olsen, M. W., and Neher, B. H.: 1948. The site of fertilization in the domestic fowl. Jour. Exper. Zool. 109:355.

Oota, Y., and Kobayashi, H.: 1962. Fine structures of the median eminence and pars nervosa of the pigeon. Annot. Zool. Jap. 35:128.

Ostmann, O. W., Ringer, R. K., and Tetzlaff, M.: 1963. The anatomy of the feather follicle and its immediate surroundings. Poultry Sci. 42:958.

Pak Poy, R. K. F., and Robertson, J. S.: 1957. Electron microscopy of the avian renal glomerulus. Jour. Biophys. Biochem. Cytol. 3:183.

Parker, G. H.: 1930. The ciliary systems in the oviduct of the pigeon. Proc. Soc. Exper. Biol. Med. 27:704.

————: 1931. The passage of sperms and eggs through the oviduct of terrestrial vertebrates. Phil. Trans. Roy. Soc. London, Ser. B. 219:381.

Parker, J. E.: 1962. Reproductive physiology in poultry. In Hafez, E. S. E., Reproduction in Farm Animals. Lea and Febiger, Philadelphia. 12:206.

————, McKenzie, F. F., and Kempster, H. L.: 1942. Fertility in the male domestic fowl. Bul. Univ. Missouri Agr. Exp. Sta. No. 347, 50 pp.

Parker, W. K.: 1888–94. On the morphology of the Gallinaceae. Trans. Linn. Soc. London, Ser. 2, 5:213.

Payne, F.: 1942. The cytology of the anterior pituitary of the fowl. Biol. Bul. 82:79.

————: 1957. A cytological study of the thyroid glands of normal and experimental fowl, including interrelationships with the pituitary, gonads and adrenals. Jour. Morph. 101:89.

————: 1959. Cytologic evidence of secretory activity in the neurohypophysis of the fowl. Anat. Rec. 134:433.

————, and Breneman, W. R.: 1952. Lymphoid areas in endocrine glands of fowl. Poultry Sci. 31:155.

Pensa, A.: 1907. Della struttura e delio sviluppo dei gangli linfatici degli uccelli (*Anser domesticus*). Ricerche fatti nel laboratorio di anatomica normale della R. Univ. di Roma ecc. 12:281.

Permkopf, E.: 1937. Vorderdarm. B Die vergleichung der verschiedenen Formtypen des Vorderdarmes der Kranioten. In Bolk, L., Göppert, E., Kallius, E., and Lubosch, W., Handbuch der vergleichenden Anatomie der Wirbeltiere, Urban and Schwarzenberg, Berlin and Vienna. 3:477.

————, and Lehner, J.: 1937. Vorderdarm. A. Vergleichenden Beschreibung des Vorderdarmes bei den einzelnen Klassen der Kranioten. In Bolk, L., Göppert, E., Kallius, E., and Lubosch, W., Handbuch der vergleichenden Anatomie der Wirbeltiere, Urban and Schwarzenberg, Berlin and Vienna. 3:349.

Petrén, T.: 1926. Die Coronararterien des Vogelherzens. Morph. Jahrb. 56:239.

Pieper, J.: 1928. On the evolution of the vertebral column in birds, illustrated by development in *Larus* and *Struthio*. Phil. Trans. Roy. Soc. London. B:216 285.

Plate, L. H.: 1924. Ueber Drüsen und Lymphknoten in der Ohrfalte der Truthenne und des Auerhahns. Arch. f. mikros. Anat. 91:208.

Portmann, A.: 1950. Les organes de la circulation sanguine. In Grassé, P.-P., Traité de Zool. Oiseaux. Masson et Cie., Paris 15:243.

————: 1961a. Sensory organs: skin, taste and olfaction. In Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:37.

Portmann, A.: 1961b. Sensory organs: equilibration. In Marshall, A J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:49.

———, and Stingelin, W.: 1961. The central nervous system. In Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:1.

Pumphrey, R.: 1961a. Sensory organs: vision. In Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:55.

———: 1961b. Sensory organs: hearing. In Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 2:69.

Pycraft, W. P.: 1898. A contribution towards our knowledge of the morphology of the bird owls Part I Pterylography. Trans. Linn Soc. London 7:223.

Rahn, H., and Painter, B. T.: 1941. A comparative histology of the bird pituitary. Anat. Rec. 79:297.

Renzoni, A., and Quay, W. B.: 1963. Comparative studies of pineal structure and composition in birds Am. Zool. 3:554.

Reynolds, S. H.: 1913. The Vertebrate Skeleton. Cambridge Univ. Press, xvi + 535 pp.

Riddle, O.: 1927. The cyclical growth of the vesicula seminalis in birds is hormone controlled. Anat. Rec. 37:1.

———, and Frey, P.: 1925. The growth and age involution of the thymus in male and female pigeons. Am. Jour. Physiol. 71:413.

Ringer, R.: In press. Thyroids In Sturkie, P. D., Avian Physiology. 2nd ed. Comstock Publ. Assoc., Ithaca, New York.

Romanoff, A. L.: 1960 The Avian Embryo. Macmillan Co., N.Y. xvi + 1305 pp.

Romer, A. S.: 1927 The development of the thigh musculature of the chick. Jour. Morph. and Physiol. 43:347.

Rooth, J.: 1953 On the correlation between the jaw muscles and the structure of the skull in Columba palumbus palumbus L. I Koninkl. Nederl. Akad. Van Wetenschappen — Amsterdam. Proc., Ser. C. 56:251.

Rosenberg, L. E.: 1941. Microanatomy of the duodenum of the turkey. Hilgardia 13:625.

Salt, W. R.: 1954. The structure of the cloacal protuberance of the vesper sparrow (Pooecetes gramineus) and certain other passerine birds. Auk 71:64.

Schauder, W.: 1923 Anatomie der Hausvögel. In Martin's Lehrbuch der Anatomie der Haustiere. Schickhardt and Ebner, Stuttgart. (Quoted from Calhoun, 1954).

Schildmacher, H.: 1931. Untersuchungen über die Funktion der Herbstschen Körperchen. Jour. Ornith. 79:374.

Scothorne, R. J.: 1959 The nasal glands of birds' a histological and histochemical study of the inactive gland in the domestic duck. Jour. Anat. 93:246.

Seaman, A. R., and Himstadt, T. H.: 1963 Correlated ultrafine structural changes of avian pecten oculi and ciliary body of Gallus domesticus: Preliminary observations on the physiology: 1. Effects of decreased intraocular pressure induced by intravenous injection of acetazolamide (Diamox). Am. Jour. Ophthal. 56:278.

———, and Storm, H.: 1963 A correlated light and electron microscope study on the pecten oculi of the domestic fowl (Gallus domesticus). Exper. Eye Res 2:163.

Shufeldt, R. W.: 1890. The Myology of the Raven. Macmillan Co., London and N.Y. xix + 343 pp.

Sinha, D., Ray, I., and Ghosh, A.: 1959. The influence of sex and maturity on the histologic structure of suprarenal glands of the pigeon. Nucleus 2 171.

Smith, M. L.: 1941. Anatomy of the brain and cranial nerves of the turkey. Univ. Colo. Stud. (Ser. A) 26:155.

Spanner, R. 1925. Der Pfortaderkreislauf in der Vogelniere. Morph. Jahrb. 54:560.

Sperber, L: 1960. Excretion. In Marshall, A. J., Biology and Comparative Physiology of Birds. Academic Press, N.Y. 1:469.

Stammer, A.: 1961. Untersuchungen über die Struktur und die Innervation der Epiphyse bei Vögeln. Acta. Biol. 7:65.

Starck, D., and Barnikol, A.: 1954. Beiträge zur Morphologie der Trigeminus-muskulatur der Vögel (Besonders der Accipitres, Cathartidae, Striges und Anseres). Morph. Jahrb. 94:1. 55 221.

Steiner, H.: 1917. Das Problem der Diastataxie des Vogelflügels. Jena. Zeitschr. f. Naturw. 55 221.

Stettenheim, P., Lucas, A M., Denington, E. M., and Jamroz, C.: 1963. The arrangement and action of the feather muscles in chickens. Proc. 13th Intern. Ornith. Cong. 2:918.

Stingelin, W.: 1958. Vergleichend morphologische Untersuchungen am Vorderhirn der Vögel auf cytologischer und cytoarchitektonischer Grundlage. Helbing & Lichtenhahn, Basel. 123 pp.

Sturkie, P. D.: 1954. Avian Physiology. Comstock Publ. Assoc., Ithaca, N.Y. xx + 423 pp.

Sullivan, G. E: 1962 Anatomy and embryology of the wing musculature of the domestic fowl (Gallus) Australian Jour. Zool. 10:458

Surface, F. M.: 1912 The histology of the oviduct of the domestic hen. Maine Agr. Exp. Sta Ann. Rep. Bul 206:395.

Technau, G.: 1936. Die Nasendrüse der Vögel zugleich ein Beitrag zur Morphologie der Nasenhöhle. Jour. Ornith. 84:511.

Terni, T.: 1925. Sur l'évolution et la structure du corps ultimobranchial chez le poulet. Comp. Rend. Assoc. Anat. 20:364.

Thompson, F. D.: 1910. The thyroid and parathyroid glands throughout vertebrates, with observations on some other closely related structures. Phil. Trans. Roy. Soc. London 201:91.

Uchiyama, T.: 1928. Zur Frage der Vv. minimae Thebesii und der Sinusoide beim Hühnerherzen. Morph. Jahrb. 60:296.

van Tienhoven, A., and Juhasz, L.: 1962. The chicken telencephalon, diencephalon and mesencephalon in stereotaxic coordinates. Jour. Comp. Neur. 118:185.

Van Tyne, J., and Berger, A. J.: 1959. Fundamentals of Ornithology. John Wiley & Sons, N.Y. xi + 624 pp.

Verma, O. P., and Cherms, F. L.: 1964. Observations on the oviducts of turkeys. Avian Dis. 8:19.

Watabe, M.: 1960. Comparative anatomy of bony labyrinth of Gallus gallus domesticus. Hirosaki Med. Jour. 11:320.

Watanabe, T.: 1960. Comparative and topographical anatomy of the fowl. VII. On the peripheral course of the vagus nerve in the fowl. Jap. Jur. Vet. Sci. 22:145.

Watzka, M.: 1939. "Weisse" und "rote" muskeln. Morph. Jahrb. 45:668.

Wentworth, B. C., and Mellen, W. J. 1961. Circulating thyroid hormones in domestic birds. Poultry Sci. 40:1275.

Westpfahl, U.: 1961. Das Arteriensystem des Haushuhnes. Wiss. Zeitschr. Humboldt–Univ. Berlin, Math.-Nat. R., 10:93.

Wetherbee, D. K.: 1957. Natal plumages and downy pteryloses of passerine birds of North America. Bul. Am. Mus. Nat. Hist. 113:339.

Williams, J. L.: 1942. The development of cervical vertebrae in the chick under normal and experimental conditions. Am. Jour. Anat. 71:153.

Wingstrand, K. G.: 1951. The Structure and Development of the Avian Pituitary from a Comparative and Functional Viewpoint. C. W. K. Gleerup, Lund, Sweden. 316 pp.

Winkelmann, R. K.: 1960. Nerve Endings in Normal and Pathologic Skin. Charles C Thomas, Springfield, Ill. viii + 195 pp.

———, and Myers, T. T., III: 1961. The histochemistry and morphology of the cutaneous sensory end-organs of the chicken. Jour. Comp. Neur. 177:27.

Wolfson, A.: 1952. The cloacal protuberance—A means for determining breeding condition in live male passerines. Bird-Banding 23:159.

Wood, C. A.: 1917. The Fundus Oculi of Birds. Lakeside Press, Chicago, Ill. 181 pp.

Wortham, R. A.: 1948. The development of the muscles and tendons in the lower leg and foot of chick embryos. Jour. Morph. 83:105.

Yamamoto, Y.: 1960. Comparative histological studies of the thyroid gland of lower vertebrates. Okajimas Fol. Anat. Jap. 34:353.

Yasuda, M.: 1960. Comparative and topographical anatomy of the fowl. III. On the nervous supply of the thoracic limb in the fowl. Jap Jour. Vet. Sci. 22:89.

———: 1961. Comparative and topographical anatomy of the fowl. XI. On the nervous supply of the hind-limb. Jap. Jour. Vet. Sci. 23.145.

Yntema, C. L.: 1943. Experiments on the origin of the sensory ganglia of the facial nerve in the chick. Jour. Comp. Neurol. 81:147.

———, and Hammond, W. S.: 1945. Depletions and abnormalities in the cervical sympathetic system of the chick following extirpation of neural crest. Jour. Exper. Zool. 100:237.

Zarrow, M. X., Greenman, D. L. Koltas, J., and Dalrymple, D.: 1962. The pituitary-adrenal axis in the bird. Gen. and Comp. Endocrinology 2:177.

Zusi, R. L.: 1962. Structural Adaptations of the Head and Neck in the Black Skimmer Rynchops nigra Linnaeus. Nuttall Ornith. Club. No. 3, Cambridge, Mass. viii + 101 pp.

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2

Digestion

When philosophical speculation began to be replaced by scientific experiment as the basis of biological knowledge, the earliest experiments on digestion were conducted with birds Reamur in 1752 published an account of his studies with kites, which he used because they regurgitate indigestible food residues from the stomach. He fed them various foods in metal tubes with open screened ends and, on recovering them, observed that meat completely disappeared but whole grains resisted digestion. He collected gastric juice by putting sponges in such tubes into the stomach and observed the action of gastric juice on meat *in vitro*. Further experiments using similar methods with carnivorous birds and other animals were conducted by Spallanzini some twenty-five years later. McCollum (1957) in his book, *A History of Nutrition*, describes these fascinating early studies and others in the development of knowledge of digestion. Although digestion has been a very active

field of physiological, biochemical, and nutritional research for many years, concepts have been changed markedly in recent years, and the processes are not yet fully understood. Some of the distinctive aspects of digestion in domestic birds are well recognized, but much of our present understanding of the process in birds is based on the general concepts derived from investigations with other species.

The main function of the digestive system is the reduction of complex food materials by hydrolysis to component water soluble (or water-miscible) units in preparation for absorption and metabolism. This is achieved by a complement of digestive enzymes which catalyze the stepwise hydrolysis of food materials in the controlled environments maintained in the various parts of the system. Viewed in its simplest terms, the digestive tract is a tube into which food is inserted at one end, from which the nutritionally useful por-

TABLE 2.1
THE DIGESTIVE ENZYMES, THEIR LOCATION AND ACTION

Enzymes	Produced by	Location of Action	Action of Enzyme
Carbohydrate-splitting			
Salivary amylase	Salivary glands	Crop	Split starch to polysaccharide and maltose units by hydrolyzing 1, 4-glucoside bonds within the starch molecule
Pancreatic amylase	Pancreas	Duodenum, small intestine	
Biliary amylase	Liver	Duodenum, small intestine	
Intestinal amylases	Small intestine	Small intestine	
Oligo-1, 6-glucosidase			Split branched-chain polysaccharides at 1,6-glucoside bonds
Maltase			Hydrolyze maltose to glucose
Sucrase			Hydrolyze sucrose to glucose and fructose
Lactase	Crop	?	Hydrolyze lactose to glucose and galactose
Protein-splitting			
Gastric protease (pepsin)	Proventriculus	Gizzard	Hydrolyze proteins at linkages containing an aromatic amino acid
Pancreatic proteases			
Trypsin	Pancreas	Duodenum, small intestine	Hydrolyze proteins at linkages containing base amino acids
Chymotrypsins	Pancreas	Duodenum, small intestine	Hydrolyze proteins at linkages of neutral amino acids
Peptidases			
Carboxypeptidase	Pancreas	Duodenum, small intestine	Hydrolyze peptides to amino acids progressively from free carboxyl end
Aminopeptidases	Small intestine	Small intestine	Hydrolyze peptides to amino acids progressively from free amino end
Dipeptidases	Small intestine	Small intestine	Hydrolyze specific peptides to amino acids
Fat-splitting			
Pancreatic lipase	Pancreas	Duodenum, small intestine	Hydrolyze triglyceride, mainly to monoglyceride and fatty acids
Phosphatidases	Pancreas	Duodenum, small intestine	Hydrolyze phospholipids to glycerol, fatty acids, base and phosphate

tion is absorbed, and the unutilized (indigestible) food components are discharged at the other end. Actually the digestion and absorption of foods is a dynamic and complex process which involves a high level of metabolic activity and turnover by the gut. Although the enzymes which catalyze the hydrolysis of food components are effective in very small amounts, the secretions in which they are contained constitute a large flow. For instance, the diet proteins as they are digested to amino acids are diluted as much as sevenfold by the proteins of the secretions of pancreas and small intestine, which in turn are largely digested and recovered. The total flow of amino acids is very much greater than that

represented by the net amount of dietary protein which is digested and absorbed (Nasset and Ju, 1961).

It is estimated that the turnover time of intestinal epithelium in experimental mammals and man is of the order of 1.5–2 days, which meant that the entire lining of the small intestine is completely replaced in this short time. Another evidence of the dynamic nature of the over-all process is the fact that for most nutrients an active process of absorption is involved, rather than a simple diffusion of substances across the wall of the absorbing cell of the intestine. Consequently, the rate of absorption is high for substances that are actively transported, and mechanisms thereby exist for selective

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[60]

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absorption. The complexity of the reaction mixtures in the gut has other nutritional implications as well, and it is known that the availability of some nutrients, especially minerals, can be markedly influenced by composition of the diet.

Knowledge of the digestive machinery and its functioning in a given species is based on (1) the enzyme apparatus, and the physiological environment in which it functions; (2) the properties of food materials which are processed, including the susceptibility of their components to enzymatic hydrolysis and the action of inhibitory substances which the food may contain; and (3) the total processing capacity of the tract. Each of the aspects will be considered in this discussion of digestion in birds. Limited consideration will be given to anatomical structure and relationships. For more detail the reader is referred to Chapter 1 on anatomy in this volume. Further sources are an extensive discussion by Farner (1960) and an authoritative review and study on microanatomy by Calhoun (1954). Selected general references which reflect current concepts based on modern studies in biochemical and physiological mechanisms of digestion and absorption are grouped in a special section of the references

THE ENZYMATIC MACHINERY OF DIGESTION

The complement of digestive enzymes considered to be present in the digestive system of chickens and other birds is shown in Table 2.1, which summarizes their location and action. The biochemical characteristics of the avian enzymes, to establish their detailed mechanisms of action, are not so well known as the more thoroughly studied mammalian enzymes.

However, the general functioning of the avian digestive system, its efficiency of digestion, the *in vitro* action of the various digestive secretions, and the effects of enzyme inhibitors which have been studied, all indicate that the avian system has the characteristics common to nonruminants generally. In the discussions to follow, particular reference will be made to information known specially to apply to poultry, but most of the material on the action of digestive enzymes has come from investigations with mammals.

The environment in which the digestive enzymes act is controlled physiologically, and is affected little by diet except under rather extreme conditions. The typical pH conditions in the various sections of the tract are shown in Table 2.2. These differ from the typical conditions in mammals by (1) less acid stomach contents, (2) somewhat more acid intestinal contents, and (3) an acid bile.

Carbohydrate Digestion

The main carbohydrate in the diet of birds is starch, supplied by grains or grain by products. The over-all process is indicated in the following equation which depicts the hydrolysis of starch to glucose, the unit compound absorbed into the blood and transported to the cells of the body.

This oversimplified description of the digestion of starch to glucose shows that it proceeds by hydrolysis, and that for every 162 grams of starch hydrolyzed there are produced 180 grams of glucose. There is essentially no change in the gross energy value in this process; although glucose has a lower energy value per gram this is a result simply of the increase in weight on hydrolysis, and the total amount of combustible energy in the equivalent amounts

$$(C_6H_{10}O_5)n \xrightarrow[\text{alpha-amylase}]{.5n \ H_2O} 5nC_{12}H_{22}O_{11} \xrightarrow[\text{maltase}]{.5n \ H_2O} nC_6H_{12}O_6$$

starch
molecular weight 162n
energy = 4.18 kcal/gm
= 677 kcal/162 gm

maltose

glucose
molecular weight 180n
energy = 3.74 kcal/gm
= 673 kcal/180 gm

TABLE 2.2

THE pH ENVIRONMENT OF THE DIGESTIVE TRACT OF FIVE SPECIES OF BIRDS*

	Chicken	Turkey	Duck	Pheasant	Pigeon
Crop	4.5	6.0	4.9	5.8	4.3
Proventriculus	4.4	4.7	3.4	4.7	4.8
Gizzard	2.6	2.2	2.3	2.1	2.0
Duodenum	5.7-6	5.8-6.5	6.0-6.2	6.0-6.6	5.2-5.4
Small intestine	5.8-6.4	6.7-7	6.1-6.9	6.2-6.8	5.3-5.9
Large intestine	6.3	6.5	6.7	6.6	5.4
Ceca	5.7	5.9	5.9	5.4	—

*Adapted from Farner (1942)

of starch and glucose is virtually the same.

Four kinds of enzymes participate in the stepwise hydrolysis of carbohydrates. The chicken produces saliva which contains amylase (carbohydrate-splitting) activity (Leasure and Link, 1940). The action of this amylase occurs in the crop during any temporary storage of food there. This action probably stops in the gizzard because of the low pH. Further carbohydrate digestion is carried on in the intestine by the amylase of pancreatic juice. Both the salivary and pancreatic amylases are alpha-amylases which attack the starch molecule by hydrolysis of random 1,4-glucoside linkages within the molecule, producing successively smaller polysaccharide units. Starch molecules consisting of straight chain (amylose) polymers have only the 1,4-linkages and can be completely degraded to the disaccharide maltose ($C_{12}H_{22}O_{11}$) by the action of these two amylases. A part of the starch in grains has a branched chain structure termed amylopectin which cannot be completely hydrolyzed by the salivary and pancreatic alpha-amylases. It requires another pancreatic enzyme termed 1,6-oligoglucosidase which specifically hydrolyzes the branching linkages, and allows the further degradation of the starch to maltose. Maltose is hydrolyzed to glucose by an intestinal enzyme called maltase. It is yet uncertain whether this enzyme acts in the intestinal lumen, or whether the final degradation of maltose to glucose takes place in the cells of the intestinal mucosa.

Specific enzymes for the hydrolysis of other disaccharides are also present in the digestive tract. They include sucrase which hydrolyzes sucrose, and lactase which hydrolyzes lactose and has been shown to be present in the crop (Hamilton and Mitchell, 1924).

Since domestic birds are grainiferous animals, it is pertinent to note that grains and other plant materials contain amylases which are active under appropriate conditions of moisture and pH. These enzymes are beta-amylases which differ from the alpha-amylase of animals in that they split maltose units from the ends of starch molecules rather than hydrolyzing interior linkages. Digestion by plant amylase action could occur in the crop or in the intestine.

Protein Digestion

The end products of protein digestion are the amino acids, which are the unit components of the highly complex protein molecules. The degradation is achieved in a stepwise fashion, with the participation of enzymes from gastric juice, pancreatic juice, and the intestinal secretions. From a nutritional standpoint, there is no significant absorption of intact protein molecules or peptides. In making this statement, it is recognized that in very young animals there is absorption of intact proteins or protein subunits from the gut, and that this is an important mechanism for acquisition of antibodies; furthermore, it is recognized that absorption of antigenic amounts of proteins is involved in allergies. From an over-all view of protein nutrition, however, these are of minor magnitude and will not be considered further in this discussion.
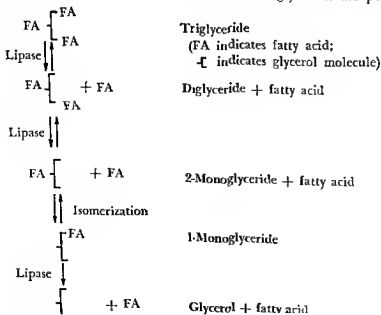
The various proteases, as shown in Table 2.1, have a high degree of specificity

absorption. The complexity of the reaction mixtures in the gut has other nutritional implications as well, and it is known that the availability of some nutrients, especially minerals, can be markedly influenced by composition of the diet.

Knowledge of the digestive machinery and its functioning in a given species is based on (1) the enzyme apparatus, and the physiological environment in which it functions; (2) the properties of food materials which are processed, including the susceptibility of their components to enzymatic hydrolysis and the action of inhibitory substances which the food may contain; and (3) the total processing capacity of the tract. Each of the aspects will be considered in this discussion of digestion in birds. Limited consideration will be given to anatomical structure and relationships. For more detail the reader is referred to Chapter 1 on anatomy in this volume. Further sources are an extensive discussion by Farner (1960) and an authoritative review and study on microanatomy by Calhoun (1954). Selected general references which reflect current concepts based on modern studies in biochemical and physiological mechanisms of digestion and absorption are grouped in a special section of the references.

THE ENZYMATIC MACHINERY OF DIGESTION

The complement of digestive enzymes considered to be present in the digestive system of chickens and other birds is shown in Table 2.1, which summarizes their location and action. The biochemical characteristics of the avian enzymes, to establish their detailed mechanisms of action, are not so well known as the more thoroughly studied mammalian enzymes.

However, the general functioning of the avian digestive system, its efficiency of digestion, the *in vitro* action of the various digestive secretions, and the effects of enzyme inhibitors which have been studied, all indicate that the avian system has the characteristics common to nonruminants generally. In the discussions to follow, particular reference will be made to information known specially to apply to poultry, but most of the material on the action of digestive enzymes has come from investigations with mammals.

The environment in which the digestive enzymes act is controlled physiologically, and is affected little by diet except under rather extreme conditions. The typical pH conditions in the various sections of the tract are shown in Table 2.2. These differ from the typical conditions in mammals by (1) less acid stomach contents, (2) somewhat more acid intestinal contents, and (3) an acid bile.

Carbohydrate Digestion

The main carbohydrate in the diet of birds is starch, supplied by grains or grain by-products. The over-all process is indicated in the following equation which depicts the hydrolysis of starch to glucose, the unit compound absorbed into the blood and transported to the cells of the body.

This oversimplified description of the digestion of starch to glucose shows that it proceeds by hydrolysis, and that for every 162 grams of starch hydrolyzed there are produced 180 grams of glucose. There is essentially no change in the gross energy value in this process; although glucose has a lower energy value per gram this is a result simply of the increase in weight on hydrolysis, and the total amount of combustible energy in the equivalent amounts

$$(C_6H_{10}O_5)_n \xrightarrow[\text{alpha amylase}]{.5n\ H_2O} 5nC_{12}H_{22}O_{11} \xrightarrow[\text{maltase}]{.5n\ H_2O} nC_6H_{12}O_6$$

starch
molecular weight 162n
energy = 4.18 kcal/gm
= 677 kcal/162 gm

maltose

glucose
molecular weight 180n
energy = 3.74 kcal/gm
= 673 kcal/180 gm

TABLE 2.2
THE pH ENVIRONMENT OF THE DIGESTIVE TRACT OF FIVE SPECIES OF BIRDS*

	Chicken	Turkey	Duck	Pheasant	Pigeon
Crop	4.5	6.0	4.9	5.8	4.3
Proventriculus	4.4	4.7	3.4	4.7	4.8
Gizzard	2.6	2.2	2.5	2.1	2.0
Duodenum	5.7–6	5.8–6.5	6.0–6.2	5.6–6	5.2–5.4
Small intestine	5.8–6.4	6.7–7	6.1–6.9	6.2–6.8	5.5–5.9
Large intestine	6.3	6.5	6.7	6.6	5.4
Ceca	5.7	5.9	5.9	5.4	—

*Adapted from Farner (1942)

of starch and glucose is virtually the same.

Four kinds of enzymes participate in the stepwise hydrolysis of carbohydrates. The chicken produces saliva which contains amylase (carbohydrate-splitting) activity (Leasure and Link, 1940). The action of this amylase occurs in the crop during any temporary storage of food there. This action probably stops in the gizzard because of the low pH. Further carbohydrate digestion is then carried on in the intestine by the amylase of pancreatic juice. Both the salivary and pancreatic amylases are alpha-amylases which attack the starch molecule by hydrolysis of random 1,4-glucoside linkages within the molecule, producing successively smaller polysaccharide units. Starch molecules consisting of straight chain (amylose) polymers can have only the 1,4-linkages and can be completely degraded to the disaccharide maltose ($C_{12}H_{22}O_{11}$) by the action of these two amylases. A part of the starch in grains has a branched chain structure termed amylopectin which cannot be completely hydrolyzed by the salivary and pancreatic alpha-amylases. It requires another pancreatic enzyme termed 1,6-oligoglucosidase which specifically hydrolyzes the branching linkages, and allows the further degradation of the starch to maltose. Maltose is hydrolyzed to glucose by an intestinal enzyme called maltase. It is yet uncertain whether this enzyme acts in the intestinal lumen, or whether the final degradation of maltose to glucose takes place in the cells of the intestinal mucosa.

Specific enzymes for the hydrolysis of other disaccharides are also present in the digestive tract. They include sucrase which hydrolyzes sucrose, and lactase which hydrolyzes lactose and has been shown to be present in the crop (Hamilton and Mitchell, 1924).

Since domestic birds are grainiferous animals, it is pertinent to note that grains and other plant materials contain amylases which are active under appropriate conditions of moisture and pH. These enzymes are beta-amylases which differ from the alpha-amylase of animals in that they split maltose units from the ends of starch molecules rather than hydrolyzing interior linkages. Digestion by plant amylase action could occur in the crop or in the intestine.

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The various proteases, as shown in Table 2.1, have a high degree of specificity

in the kinds of peptide linkages which they hydrolyze. The gastric juice contains the gastric protease (pepsin) and hydrochloric acid. It is secreted by the true stomach (proventriculus) and carries on its action primarily in the gizzard. Its action is to hydrolyze proteins at peptide linkages which involve an aromatic amino acid. The action of gastric juice is followed by the pancreatic proteases, trypsin and chymotrypsins, contained in pancreatic juice which enters the small intestine at the distal end of the duodenum. These enzymes also hydrolyze specific peptide linkages as indicated in Table 2.1. By this time the proteins have been reduced to smaller and more soluble polypeptide fragments. They are hydrolyzed to amino acids by three kinds of enzymes. One, from the pancreas, termed carboxypeptidase, hydrolyzes proteins and peptides progressively from the free acid end of the molecule. Other enzymes classed as aminopeptidases, produced by the tissues of the small intestine, conduct a similar progressive hydrolysis beginning from the end of the molecule with the free amino group. Other specific intestinal enzymes split specific dipeptides.

Fat Digestion

Hydrolysis of fats is catalyzed by the pancreatic lipase (fat-splitting enzyme) in a process which is in some ways more complex than the digestion of either carbohydrates or proteins. The pancreatic and bile secretions establish an intestinal pH of approximately 6 in which environment the bile acids emulsify the dietary fats so that they present a large surface for lipase action. The lipase catalyzes the partial hydrolysis of triglyceride fats, splitting off fatty acids by hydrolyzing the primary (alpha) glyceride ester linkages. The end product of this hydrolysis is mainly fatty acids and the 2-monoglyceride ester, because the pancreatic lipase is specific for the hydrolysis of the terminal (alpha) ester linkages (Mattson and Beck, 1956). However, part of the fat is hydrolyzed only to the diglyceride stage, and a small amount is hydrolyzed completely to fatty acids and glycerol; the complete hydrolysis apparently occurs because the 2-monoglyceride isomerizes to form 1-monoglyceride, which can be attacked by the lipase. The following is a scheme of triglyceride hydrolysis by pancreatic lipase:

The monoglycerides are powerful solu-

Triglyceride
(FA indicates fatty acid;
⊦ indicates glycerol molecule)

Diglyceride + fatty acid

2-Monoglyceride + fatty acid

1-Monoglyceride

Glycerol + fatty acid

bilizing substances, with the result that the combination of monoglycerides, diglycerides, bile salts, and fatty acids, together with some undigested triglyceride forms a highly stable fat-in-water unit known as a micelle (Hofmann and Borgstrom, 1962). This is similar to the action of detergents in solubilizing many substances. It is in the micelle form that the fat is absorbed by the intestinal cells. Within the cells there is resynthesis of the components of the mixture into triglyceride fat which is then transported to body cells. There is considerable controversy as to the proportion of the triglyceride fat which is hydrolyzed to the monoglyceride form in digestion. The weight of present evidence supports the view that the hydrolysis of a highly absorbable fat to monoglyceride and fatty acids is essentially complete, and only a small proportion of the fat is absorbed as triglyceride. This is based on the analysis of intestinal contents during digestion *in vivo*, and on experiments using fats labelled with radioisotopes which have shown extensive redistribution of fatty acids in the absorbed fat as compared to the ingested fat. This is also consistent with the finding that unabsorbed fat appears in the feces as fatty acids and soaps, rather than as glycerides.

The Control of Enzyme Secretion

The glands which secrete the enzymes and digestive juices are protected from self-digestion mainly because the enzymes are present in them in inactive forms. In the secretory cells of the pancreas, for instance, the enzymes are contained in small granules known as zymogen granules. The enzymes, which are proteins, require a chemical activation which usually involves either hydrolysis of a particular peptide bond in its structure, or the splitting out of an inactive fragment (polypeptid) of the molecule. The activation of the protein-splitting enzymes is well understood, and can serve to illustrate the principle. The gastric protease, pepsin, is secreted as pepsinogen and is activated by hydrochloric acid which is produced by separate cells. Pepsin also activates pepsinogen and the process is therefore autocatalytic. The pancreatic proteases are secreted in the inactive forms trypsinogen and chymotrypsinogen. An intestinal enzyme, enterokinase, activates trypsinogen to form trypsin, which in turn activates more trypsinogen and also chymotrypsinogen. In addition to confining the inactive enzymes in zymogen granules, the cells are equipped with enzyme inhibitors, the purpose of which is evidently to prevent damage from chance release of active enzymes within the cell.

The secretion of the various digestive juices is controlled in mammals by hormones which are produced by the gut tissues in response to the presence of food. Gastrin is produced by stomach tissue, and causes the secretion of gastric juice. Four hormones are produced by the intestinal tissues. Cholecystokinin causes gallbladder contraction to produce bile flow. Secretin stimulates pancreatic juice flow, causing secretion of juice high in sodium bicarbonate and low in enzyme activity; pancreozymin causes secretion of enzyme-rich pancreatic juice. Enterogastrone acts to inhibit flow of chyme from stomach to intestine, and acts as a rate regulator. It is likely that mechanisms analogous to these regulate digestive secretions in birds as well.

THE ORGANS OF DIGESTION AND ABSORPTION

The special features of the digestive tract of birds are easily distinguished and are related to feeding behavior and digestive efficiency. A brief description of the parts, their general functions, and particular characteristics form this section of the discussion.

Mouth

The mouth is hard surfaced, lacks teeth, and has a relatively sparse supply of salivary glands (Leasure and Link, 1940)

digestive secretions. It has a keratinized lining formed by the underlying glandular layer of the organ. The grinding action of the gizzard is greatest when stones (grit) are present, and insoluble grit can be retained in the gizzard for a very long time. The efficiency of digestion is impaired when diets containing whole grains are fed in the absence of a grinding agent, but there is little effect of grit on digestibility when the diet is composed entirely of ground components (Fritz, 1937). With the commonly used meal (mash) or pelletized diets in commercial meat production, for instance, there is no significant effect of grit on growth rate, food intake, or apparent efficiency of food utilization.

Surgical removal of the gizzard does not impair digestive function or survival so long as a suitably ground ration is fed. Digestibility studies with gizzardectomized birds have shown little impairment in food utilization (Fritz et al., 1936).

Small Intestine

This part of the digestive tract is the main section for food processing and absorption. The proximal part of the small intestine forms the duodenal loop within which is situated the large and well-defined pancreas. Gastric digestion probably continues in the early part of the duodenum until the point is reached at which pancreatic secretions (3 ducts) and bile (2 ducts) enter the gut. These secretions establish an intestinal pH in the range 5.5–6.5 which inhibits the gastric protease but allows effective digestive action of the enzymes of the pancreas and intestine. All three of the major kinds of food components are attacked by the pancreatic secretions as indicated in the summary of digestive enzymes and their action presented in Table 2.1. The main task of carbohydrate hydrolysis is carried on by the pancreatic amylase, and it has been reported that the bile in the chicken also contributes amylase activity. The pancreas also furnishes the only known digestive lipases which function in the bird.

Removal of the pancreas or ligation of the pancreatic ducts causes a profound reduction in the digestion of protein and fat in the chicken. The effect on starch digestion is much less striking, the reduction being to about 70 per cent of normal digestibility. Digestion can be restored essentially to normal levels by the addition to the diet of raw beef pancreas, or presumably pancreatic tissue from other animal sources (Ariyoshi et al., 1964).

The small intestine is lined with finger like projections (villi), the surfaces of which are made up of absorbing cells and mucussecreting (goblet) cells. It has been commonly considered that the secretions of the small intestine (the so called succus entericus) contribute the enzymes which are involved in the terminal hydrolysis of carbohydrates and proteins. On the basis of relatively recent studies, it appears at least equally likely that the hydrolysis of disaccharides and small peptides may take place in the absorbing cells of the intestinal mucosa rather than in the lumen of the intestine. It is the surface cells of the villi which participate in the rapid desquamation and regeneration which makes the gut metabolism so dynamic. It is considered likely that the intestinal enzymes in the lumen of the gut come from the desquamated cells rather than from secretory glands.

Ceca

At the junction of the small and large intestines are two blind pouches which are termed ceca. In the part adjacent to the intestine, villi are present and the general structure is similar to the small intestine. In the midportion villi are less typical and less numerous, while in the typical and less numerous, while in the ceca blind ends villi are not present. The ceca evidently do not produce any digestive secretions. They may be concerned with water absorption, since the cecal contents are generally lower in moisture content than the adjacent parts of the intestine. It has often been stated that the ceca may participate in digestion of cellulose

through the action of microorganisms, but this seems to have little or no nutritional significance. Surgical removal or ligation of the ceca does not interfere with digestive functions of the tract. The size of the ceca and nature of their contents may be markedly affected by diet; in particular, feeding lactose increases the fluidity of the contents and markedly lowers the pH of the ceca.

The significance of the microbial flora of the digestive tract has been studied extensively in recent years since the discovery that feeding small amounts of antibiotics may have nutritional benefits. A favorable effect of microbial fermentation on nutrition has been demonstrated in the case of biotin (Sunde et al., 1950) and vitamin K (Nelson and Norris, 1961), both of which are synthesized by the intestinal flora and are absorbed in nutritionally significant amounts. Removal of the ceca has been shown to improve the nutritional status in respect to these vitamins when a deficient diet is fed, indicating that the flora of the ceca destroy these vitamins or inhibit their synthesis by other organisms. In these cases at least, the effects of cecal microorganisms appear to be negative rather than beneficial. Under normal circumstances, it may be that the cecal flora are essentially innocuous, and that they may constitute a buffer against invasion by more deleterious microorganisms. However, there are no known digestive functions which can be ascribed to the ceca and their removal has no deleterious effect.

Large Intestine

Birds are characterized by a short large intestine. Its structure resembles that of the small intestine and it appears to perform some absorptive functions. Water absorption is known to take place and it is probable that it functions in regulating electrolyte balance. The large intestine communicates with the cloaca, the common excretory organ into which the urinary wastes are delivered. It has been demonstrated by radiographic studies that urine enters the large intestine and that the absorption of water and salts can occur in this region. It is probable that no important digestive and absorptive function with respect to the major organic nutrients occurs in the large intestine, since apparent digestibility is similar in colostomized and intact birds.

ABSORPTION

Absorption in the mouth and crop is limited but significant, as shown by studies in which glucose absorption was demonstrated (Soedarmo et al., 1961). Interestingly, absorption from the crop is evidently selective, because the botulinus toxin was shown to be completely unabsorbed from the ligated crop (Leasure and Folz, 1940). Absorption is mainly the function of the small intestine, which is admirably constructed to perform this function. The lining of the small intestine is covered with long fingerlike projections which are called villi. The villi are lined with absorbing cells, the outer surfaces of which are made up of microscopic fingerlike projections known as microvilli. This complex surface of the intestine provides an effective absorptive area several hundredfold greater than it would be as a simple smooth rylindrical tube. The spaces between the microvilli define the limiting dimensions of particles or micelles which can be absorbed. The structure of the luminal surface of the absorbing cells possesses the mechanisms for active absorption of nutrients. The absorbing cells which form the surface of the villi are in the constant process of growth, desquamation, and renewal which makes up the dynamic turnover of intestinal tissue.

The function of the digestive process is to hydrolyze food components to their unit structures in preparation for absorption by the intestinal cells. For both carbohydrates and amino acids, there is compelling evidence to show that the process of absorption is an active one—not simply a matter of diffusion of the water-soluble compounds across the cell wall into the

TABLE 2.3
TIME REQUIRED FOR FOOD PASSAGE
IN CHICKENS AND TURKEYS

	Passage Time Hours' Minutes
Chickens	
Hens	3.46
Pullets	3.12
Cocks	3.20
Hens, high environmental temp. ...	3.46
Hens, low environmental temp.	3.51
Hens, layers	3.42
Hens, nonlayers	3.50
Turkeys	
Young hens	2.27
Hens, layers	3.13
Hens, nonlayers	4.16

intestinal mucosa. The evidence for active transport is based on observations showing that simple sugars differ markedly in the rate at which they are absorbed—for instance, xylose is absorbed by diffusion at a slow rate, while glucose is absorbed comparatively rapidly. Furthermore, galactose, glucose, and fructose, all actively absorbed, differ markedly in rate of absorption. The evidence for amino acids shows that the stereoisomers are absorbed at different rates indicating a selective and active transport for the nutritionally important amino acids. After absorption into the absorbing cells these substances are transferred into the blood stream across the base of the cell.

At the present time, it is not known whether a similar active transport system is involved in the absorption of fats. The significance of the micelle structure as the form of fat absorption seems well established, but a transport mechanism has not been defined.

A micelle size of the order of 50 mμ diameter is necessary in order to penetrate the space between two microvilli, and this is readily achieved under the conditions of the intestine. After absorption, triglyceride fat is resynthesized in the absorbing cell, and the fat droplet is ready for transport. In the mammal, this is mainly in the lymph via the thoracic duct to the jugular vein. In birds, it is not known whether the main path is via the lymphatics or the portal blood.

Different regions of the small intestine apparently are involved in the absorption of various classes of substances. The fats are absorbed in the proximal half of the small intestine. The absorption of various carbohydrates differs as to location. This regional specificity likewise suggests that specific transport mechanisms are involved for all of the important major nutrients.

NUTRITIONAL ASPECTS OF DIGESTION

The digestive tract of the chicken and other domestic birds is relatively short, and the time required for food to pass through the tract is also relatively short. Data illustrating approximate time for food passage are summarized in Table 2.3 (Hillerman *et al.*, 1953). In spite of these seeming disadvantages, the efficiency of digestion and the total capacity for the intake and processing of foods are quite high.

Chickens are typical nonruminants, as illustrated by ability to utilize various reference substances (Table 2.4). Cornstarch, sucrose, and glucose are nearly quantitatively digested and absorbed, while cellulose is completely unutilized. The utilization of these carbohydrates as they exist in natural food materials is consistent with the results obtained when they are fed as the pure substances.

Estimation of the carbohydrate value of a feeding material is frequently made on the basis of chemical analysis for crude

TABLE 2.4
THE DIGESTIBILITY OF REFERENCE SUBSTANCES
BY THE CHICKEN

	Per cent
Cornstarch	97
Sucrose	95
Glucose	97
Cellulose	0
Casein	100
Fish protein	95
Isolated soybean protein ...	87
Corn oil	93
Soybean oil	98
Beef tallow	71

fiber, which determines that portion of the organic matter of the feedstuff which is undissolved by hot acid and hot alkali. This analytical method makes an empirical estimation of the indigestible carbohydrates, including variable amounts of cellulose and hemicelluloses which form the main part of plant cell walls, and lignin which is a cementing substance of plant cell walls. Tables of digestibility of food components frequently indicate a measurable digestibility of the crude fiber fraction of feeding materials, suggesting that these complex carbohydrates are somehow digested and utilized. It is quite clear that there are no animal-produced enzymes which can hydrolyze cellulose or lignin, so that whatever modification or disappearance of these compounds apparently takes place in the digestive tract of a bird is due to the action of plant or microbial enzymes. The nutritional significance of "digestible" crude fiber is therefore open to question, and it is more likely to be due to analytical artifacts than to true utilization. When it is also recognized that the crude fiber determination generally underestimates the total indigestible carbohydrates of the feeding material, it becomes clear that this empirical estimation is of very limited utility in estimating the value of feeds for birds.

Direct determination of starch and sugar content of feedstuffs to estimate their useful carbohydrate content is a promising approach (Carpenter and Clegg, 1956) and may be developed into a more comprehensive system of feed evaluation. All chemical methods are complicated by the fact that the digestibility of a feedstuff may be influenced by the distribution of the indigestible components such as cellulose and lignin. Since these compounds make up the cell walls of plant materials, it is possible that they may protect otherwise digestible components that are "locked" within the cells. This is the basis for the view that crude fiber or indigestible carbohydrates interfere with the utilization of other nutrients, and therefore are an underestimate of the total indigestibles of the diet. The addition of pure cellulose to a diet does not reduce the utilization of other components, but is handled by the bird simply as a diluent.

Extensive investigations of the digestibility of various carbohydrates and crude fiber in natural feedstuffs have been reported by Fraps (1931) and Bolton (1955). Their findings agree with the concept that starches and sugars are highly utilized, and that cellulose and lignin have zero value. The main uncertainties are associated with estimating the utilization of hemicelluloses and pentosans which are of questionable value to the bird. Cellulose has been shown to be unutilized, both by digestion trials (Tasaki and Kibe, 1959) and in metabolizable energy studies (Anderson et al., 1958). Turkeys have been considered to be good "roughage consumers," and it was thought that they might make better use of crude fiber components than other birds. Dymsza and co-workers (1955) found very little apparent utilization of crude fiber in digestion studies with growing turkeys.

As indicated in Table 2.4, the utilization of various isolated proteins is also quite high. Furthermore, the capacity or tolerance for high dietary levels of protein is also high. There is no apparent reduction in protein digestibility when diets containing protein at twice the usual levels or even more are fed. In general, the digestibility of feedstuff proteins from plant and animal sources can be considered to be of the order of 80–85 per cent. However, the proteins classified as keratins, from such sources as hoofs, feathers, and hair, are poorly digested. Also proteins which are subjected to severe heat treatment in processing may show reduced digestibility due to the creation of chemical bonds which are resistant to attack by digestive enzymes. These effects can be detected by in vitro enzyme digestibility (such as with pepsin or trypsin), by solubility in various solutions (such as dilute alkali), or by analysis for specific chemical linkages. One

of the latter which is of particular interest is the estimation of the epsilon-amino group of lysine. In undamaged proteins, this reactive group is in the free uncombined state and can be measured by appropriate reagents; in a heat-damaged protein, this group forms indigestible linkages. A decrease in epsilon-amino lysine indicates both a decrease in the lysine value in the protein and also general damage which interferes with digestion (Carpenter, 1960).

Vegetable oils and such animal fats as lard and the fat of poultry are well utilized by chickens and other domestic birds. Furthermore, the capacity for utilization of fat is very high, and it has been shown experimentally that diets in which most or even all nonprotein calories are derived from fat can be fed successfully (Donaldson et al., 1957; Renner and Elcombe, 1963). This is in interesting contrast to the generally accepted view of only a few years ago that poultry have a very limited capacity to utilize fat. Digestibility (absorbability) data for representative fats are given in Table 2.4 and show virtually quantitative utilization of common vegetable oils and low melting animal fats. Animal fats of high melting point, such as beef tallow, are less well utilized (Renner and Hill, 1960a). Studies of the utilization of fatty acids fed as such have shown that the long chain saturated fatty acids are virtually unutilized; absorbability is higher for shorter chain saturated fatty acids. The unsaturated fatty acids, as oleic acid and linoleic acid, are highly absorbable. The absorbability of a particular fatty acid depends on the composition of the mixture in which it is fed, but in general the utilization of saturated fatty acids fed in the free form is very much less than when they are part of a triglyceride fat. This is consistent with the present concept of digestion and absorption of fats (as discussed in a previous section) in which the formation of micelles through detergent action depends on the presence of monoglycerides. In general, it is found that a

free fatty acid mixture derived from the hydrolysis of a triglyceride fat is less well utilized than the fat from which it was prepared (Renner and Hill, 1961). Recent studies have indicated that oleic acid may have special properties in facilitating the utilization of other fatty acids (Young and Garrett, 1963).

An extensive summary of the scientific literature on digestibility of feedstuffs for poultry has been prepared by Olsson (1950). It is organized according to the conventional analysis scheme for feeding materials and shows the apparent digestibility for crude protein, ether extract (fat), crude fiber, and nitrogen-free extract (carbohydrate). Digestibility studies are difficult to conduct with birds because they excrete the indigestible and urinary wastes together. Surgical alteration (such as colostomy) or chemical separation of the excreta components is necessary. The older data in the literature are particularly subject to the limitations of the methods used. The total nutrient value of the major organic components of feedstuffs can be determined as metabolizable energy (ME) by heat of combustion analysis (bomb calorimetry) using the mixed excreta (Hill et al., 1960). Extensive studies of the ME value of feedstuffs for poultry have been conducted in recent years. The values obtained agree closely with those which can be calculated from well-conducted digestion trials, showing that the total ME of a diet or diet component is the sum of the energy values of its digestible protein, carbohydrate and fat. Representative values for the ME of some common feeding materials are shown in Table 2.5.

INHIBITORY EFFECTS

Deleterious effects on digestion and absorption are associated with certain feedstuffs. Uncooked soybean meal has a marked growth retarding effect on nonruminant animals, including chicks and turkey poults, due to the presence of several growth inhibiting substances. A

TABLE 25

REPRESENTATIVE METABOLIZABLE ENERGY VALUES FOR
COMMON FEEDING MATERIALS FOR CHICKENS

Material	Metabolizable Energy*
	kcal/kg
Corn	3,370
Milo	3,300
Wheat	3,190
Barley	2,820
Oats	2,660
Wheat middlings	1,890
Wheat bran	1,300
Soybean meal, 50 per cent protein	2,500
Soybean meal, 44 per cent protein	2,210
Cottonseed meal, 44 per cent protein	1,980
Meat meal, 50-55 per cent protein	1,980
Fish meal, menhaden	2,900
Alfalfa meal, 17 per cent protein	1,370
Alfalfa meal, 20 per cent protein	1,580
Feed grade tallows	7,110
Feed grade greases	7,920
Fish oils	8,050

*All data on air-dry basis, assuming 90 per cent dry matter except for corn (88 per cent), meat and fish meals (93 per cent), and fats (moisture free)

part of the effect is due to the so called soybean trypsin inhibitor, a substance which specifically interferes with the action of the digestive enzyme trypsin. The digestibility of unheated soybeans or soybean meal by chickens is lower than for the properly cooked materials, and the inhibitors affect the utilization of both protein and fat. Although the antitrypsin substances do not entirely explain the deleterious effect of raw soybeans, they constitute an important part of the effect. Under some conditions, this can be overcome by feeding the purified enzyme in combination with the raw protein, but this is not effective under all circumstances. Subjecting the soybean meal to moist heat treatment completely destroys the inhibitory properties and allows digestion to proceed normally (Renner and Hill, 1960b; Brambila et al., 1961). Gossypol, a toxic substance present in cottonseed, can interfere with digestion as indicated by reduction in the metabolizable energy of gossypol-containing diets (Hill and Totsuka, 1964).

A growth inhibiting effect characteristic of barley produced under dry farming conditions can be overcome by supplementing the diet with plant or microbial amylase preparations or by water treatment of the barley followed by drying (Jensen et al., 1957). A highly purified bacterial enzyme, beta-glucanase, is effective in very small amounts in improving the nutritional performance of barley, suggesting that the specific glucosidal linkages (beta-1,3 and beta-1,4) attacked by this enzyme may be the limiting factors in barley utilization (Ricks et al., 1962). It has been found that water treatment and the feeding of bacterial enzymes significantly increases the metabolizable energy of barley, indicating improvement in digestibility. The various findings suggest that the poor results from feeding this kind of barley are due to a specific deleterious effect of a specific indigestible carbohydrate—not merely to the fact that the carbohydrate value of the barley is somewhat reduced because part of it is indigestible.

Perhaps a parallel situation is involved in recent studies which have shown that certain natural gums, including the gums of guar and tragacanth, markedly inhibit the growth of chicks when fed at low levels (less than 5 per cent) in the diet (Kratzer and Vohra, 1963). The mechanism by which gums are inhibitory to growth is not known, but the observations suggest interference with the digestive process, as well as more direct inhibitory effects. Enzyme treatment of the gums has been effective in overcoming inhibition.

The characteristics of carbohydrates from various plant sources, or factors associated with them, may have important effects on utilization. Corn varieties containing a high proportion of the starch in amylose (linear) form have been shown to have markedly lower digestibility of starch than the usual varieties which have less amylose and a higher proportion of amylopectin (branched) (Borchers, 1962). Whether this is due to the glucosidic linkages in the respective starches or to factors of a different nature associated with starch configuration is not known.

The digestive efficiency and digestive capacity of birds is markedly stable. The value of a diet per unit weight, measured as metabolizable energy which indicates its over-all digestion, absorption, and utilization, is essentially unaffected by the level of feeding. Even conditions such as treatment with certain hormones which markedly increase the level of food intake above normal have no effect on the percentage digestibility or metabolizable energy per unit weight of the diet. Aside from some characteristics associated with the very young chick, there is essentially no effect of age on digestive efficiency, and differences associated with breed, sex, growth rate, etc., are evidently minor and of little practical significance.

In general, associative effects of food components do not occur. That is to say, the value of a food mixture is generally the sum of the individual values of its components. However, there are some interesting and important exceptions to this generalization. For instance, there may at times be an improvement in the absorbability of poorly utilized fats (such as beef tallow) or fatty acids when they are fed in combination with a highly absorbable fat, indicating that the properties of the mixture are not directly predictable from the properties of the component parts. Another example is the interference of certain diet components with the availability of certain minerals, particularly trace minerals. Feeding a high level of calcium, for instance, can interfere with the utilization of manganese. Furthermore, the availability of zinc seems to be reduced in the presence of soybean meal due to specific interference of the combination of calcium phytate and certain protein components with the absorption of zinc (O'Dell and Savage, 1960). The availability of zinc can be enhanced under these unfavorable conditions by adding to the diet chelating agents such as EDTA (ethylene diamine tetraacetic acid) (Kratzer et al., 1959). Such substances modify the solubility and ionic properties of metal ions, and thereby influence their availability to the animal. Other natural binding agents and chelating substances undoubtedly exist in foods and their hydrolysis products which can modify the utilization of required minerals. Some amino acids, and probably protein fragments as well, have chelating properties.

Minerals are absorbed and excreted in the gut by active transport mechanisms which are under the control of the systems which maintain constant composition of body fluids and structures (homeostasis). The extent of absorption of iron, for instance, is controlled according to need for synthesis of hemoglobin. It is considered likely that the state of an iron-binding protein in the intestinal tissues determines the amount of iron absorbed. The absorption of sodium, potassium, calcium, chloride, phosphate, and sulfate all have been shown to take place by active transport.

OTHER FUNCTIONS AND PROPERTIES OF THE DIGESTIVE SYSTEM

Though the digestive and absorptive functions of the gastrointestinal tract predominate, some biochemical processing also occurs in the gut tissues. One such process is the synthesis of vitamin A [com carotenoid pigments which serve as its precursors. Another, of course, is the synthesis of triglycerides and phospholipids from the digestion products of fats.

The significance of the microflora of the gut has been studied extensively in several ways. The chick was among the first animal species to be reared under germfree conditions, demonstrating that a normal bacterial flora is not necessary to life. Since the discovery that small amounts of antibiotics added to the diet of chicks can markedly stimulate growth, many investigations of the relation of diet and environment to gut flora and the antibiotic effect have been made. The concept now generally accepted is that antibiotics exert their favorable effect by overcoming inhibitory effects of the bacterial flora of the gut, based on three kinds of evidence. First, growth of chicks reared in clean quarters, uncontaminated by previous use for chickens, is not improved by antibiotic feeding; similar chicks in contaminated quarters commonly used for chickens show marked growth stimulation by antibiotic feeding, their maximum growth rate being equal to the chicks in the clean environment. This indicates a growth retarding effect associated with environment (Coates et al., 1952). Second, the growth stimulating effect does not occur under germfree conditions (Forbes and Park, 1959). Third, the establishment of a known, controlled microflora in chicks has shown a

marked inhibitory effect from *Clostridium welchii*, which was prevented by feeding penicillin (Lev and Forbes, 1959). The degree of response to dietary antibiotics has been difficult to relate to changes in intestinal flora, and it is unlikely that a single kind of inhibitory effect is involved under all conditions, but it is clear that the antibiotics exert their effects through the microflora.

Associated with the favorable effect of antibiotics on growth is a reduction in the weight and thickness of the intestine (Coates et al., 1955). The significance of this change is not known, but it suggests the possibility of improved gut function. Recently, it was reported that dietary metabolizable energy was increased significantly by antibiotic supplementation under conditions in which the growth of chicks was improved 10–20 per cent (Nelson et al., 1963).

In a sense, the lumen of the digestive tract is part of the external environment of the animal, and is subject to the external influences of food supply and microorganisms. It is quite remarkable that the conditions in the tract are maintained so uniformly, as indicated by such measures as the pH and the percentage of water in the gut contents (Lepkovsky et al., 1957). The relationship of host to microflora in poultry includes the inhibitory effect discussed above, but there are also favorable effects. Vitamin K and biotin are synthesized by the flora and absorbed in significant amounts by the chicken. Furthermore, the established flora may serve as a buffer to prevent invasion of the gut by more unfavorable microorganisms and may therefore represent the best compromise which can be achieved by the host animal in the prevailing environment.

REFERENCES

Anderson, D. L., Hill, F. W., and Renner, Ruth: 1958. Studies of the metabolizable and productive energy of glucose for the growing chick. Jour. Nutr. 65:561.

Ariyoshi, S., Koike, T., Furuta, F., Ozone, K., Matsumura, Y., Dimick, M. K., Hunter, W. L., Wang,W., and Lepkovsky, S.: 1964. The digestion of protein, fat and starch in the depancreatized chicken. Poultry Sci. 43:232.

Bolton, W.: 1955. The digestibility of the carbohydrate complex by birds of different ages. Jour. Agr. Sci. 46:420.

: 1962. Digestion in the crop of the fowl. Proc. Nutr. Soc. 21:xxiv.

Borchers, R.: 1962. Digestibility of the starch of high-amylose corn by rats. Cereal Chem. 39:145.

Brambila, S, Nesheim, M. C., and Hill, F. W.: 1951. Effect of trypsin supplementation on the utilization by the chick of diets containing raw soybean oil meal. Jour. Nutr. 75:13.

Calhoun, M. L.: 1954. Microscopic Anatomy of the Digestive System of the Chicken. The Iowa State University Press, Ames, Iowa.

Carpenter, K. J.: 1960. Estimation of the available lysine in animal-protein foods. Biochem. Jour. 77:604.

———, and Clegg, K. M.: 1956. Metabolizable energy of poultry feeds in relation to their chemical composition. Jour. Sci. Food and Agr. 7:45.

Coates, M. E., Davies, M. K., and Kon, S. K.: 1955. The effects of antibiotics on the intestine of the chick. British Jour. Nutr. 9:110.

———, Dickinson, C. D, Harrison, G. F., Kon, S. K., Porter, J. W. G., Cummins, S. H., and Cuthbertson, W. F. J.: 1952. A mode of action of antibiotics in chick nutrition. Jour. Sci. Food and Agr. 3:43.

Donaldson, W. E., Combs, G. F., Romoser, G. L., and Supplee, W. C.: 1957. Studies on energy levels in poultry rations. 2. Tolerance of growing chicks to dietary fat. Poultry Sci. 36:807.

Dymza, H., Boucher, R. V., and McCartney, M. G.: 1955. Investigation of crude fibes digestion in 12-week-old turkeys. Poultry Sci. 34:210.

Farnes, D. S.: 1942. The hydrogen ion concentration in avian digestive tracts. Poultry Sci. 21:445.

Farner, D. W.: 1960. Digestion and digestive system. Chapter XI in Biology and Comparative Physiology of Birds, ed. by A. J. Marshall. Academic Press, New York, p. 411.

Fisher, H., and Weiss, H. S.: 1956. Feed consumption in relation to dietary bulk and energy level: the effect of surgical removal of the crop. Poultry Sci. 35 418.

Forbes, M, and Park, J. T.: 1959. Growth of germ-free and conventional chickens: effect of diet, dietary penicillin and bacterial environment. Jour. Nutr. 67 69.

Fraps, G. S.: 1931. Digestibility by chickens of the constituents of the nitrogen-free extract of feeds. Texas Agr. Exp. Sta. Bul. 437-5.

Fritz, J. C.: 1937. The effect of feeding grit on digestibility in the domestic fowl. Poultry Sci 16:75.

———, Burrows, W. H., and Titus, H. W · 1936. Comparison of digestibility in gizzardectomized and normal fowls. Poultry Sci. 15 259.

Hamilton, T. S., and Mitchell, H. H.: 1924. The occurrence of lactase in the alimentary tract of the chicken. Jour. Agr. Res 27:605.

Heuser, G. F., and Scott, M. L.: 1951. Studies in duck nutrition. 1 Methods of feeding Poultry Sci. 30:161.

Hill, F. W., Anderson, D. L., Renner, R., and Carew, L. B., Jr.: 1960. Studies of the metabolizable energy of grains and grain products for chickens. Poultry Sci. 39.573.

———, and Totsuka, K.: 1964. Studies of the metabolizable energy of cottonseed meals for chicks, with particular reference to the effects of gossypol. Poultry Sci. 43:362.

Hillerman, J. P., Kratzer, F. H., and Wilson, W. O.: 1953. Food passage through chickens and turkeys and some regulating factors. Poultry Sci. 32:332.

Hofmann, A. F., and Borgstrom, B.: 1962 Physico-chemical state of lipids in intestinal content during their digestion and absorption. Fed. Proc. 21:15.

Jensen, L. S., Fry, R. E., Allred, J. B., and McGinnis, J.: 1957. Improvement in the nutritional value of barley for chicks by enzyme supplementation. Poultry Sci. 36:919.

Kare, M. R., Black, R., and Allison, E. G.: 1957. The sense of taste in the fowl. Poultry Sci. 36:129.

Kratzer, F. H., Allred, J. B., Davis, P. N., Marshall, B. J., and Vohra, P.: 1959. The effect of autoclaving soybean protein and the addition of ethylenediaminetetraacetic acid on the biological availability of dietary zinc for turkey poults. Jour. Nutr. 68.313.

———, and Vohra, P.: 1965. The growth-depressing effect of certain naturally occurring polysaccharides for chicks. Proc. 6th Int. Cong. on Nutr., p. 122.

Leasure, E. E., and Foltz, V. D.: 1940. Experiments on absorption in the crop of the chicken. Jour. Am. Vet. Med. Assn. 96:236.

———, and Link, R. P.: 1940. Studies on the saliva of the hen. Poultry Sci. 19:131.

Lepkovsky, S., Lyman, R., Fleming, D., Nagumo, M., and Dimick, M. K.: 1957. Gastro-intestinal regulation of water and its effect on food intake and rate of digestion. Am. Jour. Physiology 188.327.

———, Chari-Bitron, A., Lyman, R., and Dimick, M. K.: 1960a. Food intake, water intake and body water regulation. Poultry Sci. 39:390.

———, Chari-Bitron, A., Lemmon, R. M, Ostwald, R. C., and Dimick, M. K.: 1960b. Metabolic and anatomic adaptations in chickens "trained" to eat their daily food in two hours. Poultry Sci. 39:385.

Lev, M., and Forbes, M.: 1959. Growth response to dietary penicillin of germ-free chicks and of chicks with a defined intestinal flora. Brit. Jour. of Nutr. 13:78.

Lindenmaier, P., and Kare. M. R.: 1959. The taste end organs of the chicken. Poultry Sci. 39:545.

McCollum, E. V.: 1957. A History of Nutrition. Houghton-Mifflin Co., Boston.

Mattson, F. H., and Beck, L. W.: 1956. The specificity of pancreatic lipase for the hydroxyl groups of glycerides. Jour. Biol. Chem. 219:735.

Nasset, E. W., and Ju, J. S.: 1961. Mixture of endogenous and exogenous protein in the alimentary tract. Jour. Nutr. 74:461.

Nelson, F. E. Jensen, L. S., and McGinnis, J.: 1963. Studies on the stimulation of growth by dietary antibiotic 2 Effect of antibiotics on metabolizable energy of the diet. Poultry Sci. 42 909.

Nelson, T. W., and Norris, L. C.: 1961. Studies on the vitamin K requirement of the chick. The effect of age and cecectomy on the vitamin K requirement of the chick. Poultry Sci. 40:592.

O'Dell, R. L., and Savage, J. E.: 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. and Med. 103:304

Olsson, Nils: 1950 Digestion Experiments on Poultry. Kungl. Lantbrukshogskolan Och Statens Lantbruksforsok, Statens Husjursforsok. Stockholm Meddlelande Nr. 45:1.

Renner, R., and Hill, F. W.: 1960a. The utilization of corn oil, lard and tallow by chickens of various ages Poultry Sci. 39.849.

——, and Hill, F. W.: 1960b Studies of the effect of heat treatment on the metabolizable energy value of soybeans and extracted soybean flakes for the growing chick. Jour. Nutr. 70:219

——, and Hill, F W.: 1961. Factors affecting the absorbability of saturated fatty acids in the chick. Jour. Nutr 74.254.

——, and Elcombe, A. M : 1963. Factors affecting the ability of the chick to utilize fatty acids in "carbohydrate-free" diets Fed Proc 22:490

Rickes, E. L., Ham, L. A., Mascatelli, A., and Ott, W. H: 1962. The isolation and biological properties of an B-glucanase from B subtilis. Arch. Biochem. and Biophys. 96:371.

Soedarmo, D, Kare, M. R., and Wasserman, R. H.: 1961. Observations on the removal of sugar from the mouth and the crop of the chicken. Poultry Sci. 40.123.

Sunde, M. L., Cravens, W. W., Elvehjem, C. A. and Halpin, J. G.: 1950. The effect of diet and cecectomy on the intestinal synthesis of biotin in the mature fowl. Poultry Sci. 29:10.

Tasaki, I., and Kibe, K.: 1959. A study on the digestion of cellulose in poultry. Poultry Sci. 38:576

Young, R. J., and Garrett, R. L.: 1963. Effect of oleic and linoleic acids on the absorption of saturated fatty acids in the chick. Jour. Nutr. 81:321.

GENERAL REFERENCES

Bloch, K Lipide Metabolism. John Wiley and Sons, Inc., New York, 1960.

Bradley, O. C., and T. Grahame. The Structure of the Fowl. Third edition. J. B. Lippincott Co., Philadelphia, 1950

Calhoun, M. L. Microscopic Anatomy of the Digestive Tract of the Chicken. The Iowa State University Press, Ames, Iowa, 1954.

Farmer, D S Digestion and the digestive system. Chapter XI in Biology and Comparative Physiology of Birds. Marshall, A. J, editor, Academic Press, New York, 1960.

Sturkie, P. D. Avian Physiology. Comstock Publishing Co, Ithaca, New York, 1954.

White, A. F. Handler, and E. L. Smith. Principles of Biochemistry. McGraw-Hill Book Co., New York, 1964.

Wilson, T. H Intestinal Absorption. W. B. Saunders Co, Philadelphia, 1962.

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3

Heredity and the Defective in Poultry

Changes in all phases of the poultry industry have been rapid and have led to changes in patterns of pathological problems. Certain salient facts are mentioned to focus attention on the biological complexity involved in production phenotypes of domestic poultry.

A major problem is the continued and considerable change in environmental conditions that man forces on his animals. The tendency is to consider egg layers, broilers, and turkeys as mass production units with machinelike performance rather than biological creatures, responsive to environmental stresses. The danger inherent in "intensivism" is that increase in stressors of all sorts can and do occur. Deep litter, no litter, slatted floor, wire floor, mechanical feeders, automatic egg-gathering devices, feeds designed to give a maximum of power for production, with high protein and high energy content, alteration in photoperiods, various kinds of confinement with their fatigue and social order prob-

lems are just a few of the "improvements" which are not entirely free of conditions contributing to pathological problems. Unfortunately the genetic adaptivity of the birds increases at a much slower rate than does the ingenuity of man in thinking up new ways to harass them.

There has been a gradual concentration of breeding stocks into a relatively few decision-making hands, so that there are few fountainhead sources of genetic material, and the resultant widespread dissemination of single source stocks has resulted in the loss of the isolation which occurs from many small breeder locations. This may be a potential means of speeding the dissemination of new or existing diseases. There has been a decided change in the type of commercial stocks with small-bodied, efficient egg-laying strains and rapid-growing, large, specialized varieties for meat production. Almost without exception the commercial bird is a result of a well-planned and tested cross. The con-

McCollum, E. V.: 1957. A History of Nutrition. Houghton-Mifflin Co., Boston.

Mattson, F. H., and Beck, L. W.: 1956. The specificity of pancreatic lipase for the hydroxyl groups of glycerides. Jour. Biol. Chem. 219:735.

Nasset, E. W., and Ju, J. S.: 1961. Mixture of endogenous and exogenous protein in the alimentary tract. Jour. Nutr. 74:461.

Nelson, F. E., Jensen, L. S., and McGinnis, J.: 1963. Studies on the stimulation of growth by dietary antibiotics. 2. Effect of antibiotics on metabolizable energy of the diet. Poultry Sci. 42:909

Nelson, T. W., and Norris, L. C.: 1961. Studies on the vitamin K requirement of the chick. The effect of age and cecectomy on the vitamin K requirement of the chick. Poultry Sci. 40:392.

O'Dell, B. L., and Savage, J. E.: 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exp. Biol. and Med. 103 304

Olsson, Nils. 1950. Digestion Experiments on Poultry. kungl. Lantbrukshogskolan Och Statens Lantbruksforsk. Statens Husjursforsok. Stockholm Meddielande Nr. 43:1.

Renner, R., and Hill, F. W.: 1960a. The utilization of corn oil, lard and tallow by chickens of various ages. Poultry Sci. 39 849.

———, and Hill, F. W.: 1960b. Studies of the effect of heat treatment on the metabolizable energy value of soybeans and extracted soybean flakes for the growing chick. Jour. Nutr. 70:219

———, and Hill, F. W.: 1961. Factors affecting the absorbability of saturated fatty acids in the chick. Jour. Nutr. 74 254.

———, and Elcombe, A. M.: 1963. Factors affecting the ability of the chick to utilize fatty acids in "carbohydrate-free" diets. Fed. Proc. 22:490.

Rickes, E. L., Ham, E. A., Mascatelli, A., and Ott, W. H.: 1962. The isolation and biological properties of an B-glucanase from B. subtilis. Arch. Biochem. and Biophys. 96:371.

Soedarmo, D., Kare, M. R., and Wasserman, R. H.: 1961. Observations on the removal of sugar from the mouth and the crop of the chicken. Poultry Sci. 40:123.

Sunde, M. L., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G.: 1950. The effect of diet and cecectomy on the intestinal synthesis of biotin in the mature fowl. Poultry Sci. 29:10.

Tasaki, I., and Kibe, K.: 1959. A study on the digestion of cellulose in poultry. Poultry Sci. 38:976

Young, R. J., and Garrett, R. L.: 1963. Effect of oleic and linoleic acids on the absorption of saturated fatty acids in the chick. Jour. Nutr. 81:321.

GENERAL REFERENCES

Bloch, K. Lipide Metabolism John Wiley and Sons, Inc., New York, 1960.

Bradley, O. C., and T. Grahame. The Structure of the Fowl. Third edition. J. B. Lippincott Co., Philadelphia, 1960.

Calhoun, M. L. Microscopic Anatomy of the Digestive Tract of the Chicken. The Iowa State University Press, Ames, Iowa, 1954.

Farner, D. S Digestion and the digestive system. Chapter XI in Biology and Comparative Physiology of Birds. Marshall, A. J editor. Academic Press, New York, 1960.

Sturkie, P. D. Avian Physiology Comstock Publishing Co., Ithaca, New York, 1954.

White, A., P. Handler, and E. L. Smith. Principles of Biochemistry. McGraw-Hill Book Co., New York, 1964.

Wilson, T. H. Intestinal Absorption. W. B. Saunders Co., Philadelphia, 1962.

strands are coiled into a helix so that particular nucleotides pair across the interstices of the helix.

Adenine-thymine and guanine-cytosine are the variable bases in DNA which are involved in the genetic code. There is evidence that messenger ribonucleic acid (RNA) is involved as a transport unit between the blueprint DNA in the nucleus and the cytoplasmic RNA associated with the ribosomes which are the site of protein syntheses. The DNA-RNA instruction code is not only the biochemical carrier of information of the life process from one generation to the next, but also determines the development of the new individual. The directions are formed from chance combinations of DNA arrangements from the sire and the dam at conception. The blueprint for reaction to various environments is set at this time for the individual. How it fares depends upon its hereditary endowments and the severity of environmental conflicts.

Trait Expression and Variation

The "gene" locus concept as a physiologically differentiated chromosomal segment is a satisfactory representation of a hereditary unit for selection theory. The smallest hereditary unit for selection purposes is the individual bird: the unit used by the poultry breeder.

The phenotype or expression of a trait results from the combined influence of hereditary messages and environmental forces. The longer the chain of events between the first action of the "gene" and its final expression in the phenotype the greater the complexity of effects which can arise from a simple alteration in the molecular configuration. Much of the complexity of relation between "gene" and character is attributed to the multiplicity of stages intervening between initial action and final expression in a series of reactions, each requiring a characteristic primary action of a "gene." Some clear examples of this type have been described in Neuro-

spora by numerous investigators beginning with Beadle and Tatum (1941), and lead to the one gene-one enzyme hypothesis. Investigators have determined that specific loci do control specific enzymes, and defects in the message system result in a breakdown of biochemical processes at a variety of levels, any of which result in functional disturbances.

Variation

Studies in several species show that each carries a considerable proportion of loci and even chromosomal segments which seem less desirable than its alternate. It might be expected that the superior allel or chromosome would supersede the other through selection so that some sort of genetic uniformity would be found for the superior allel, but polymorphism seems to be the rule in many populations rather than the exception. At one time there was a widespread opinion that selection would result in the establishment of the more favorable allel in the homozygous state and produce the ultimate animal. However genetic variation in a population seems necessary for adaptivity. Lerner (1954) cites considerable evidence that continual selection and/or inbreeding (both result in a loss of heterozygosity) in populations of chickens almost invariably results in loss of fitness and eventually an attenuation of progress in selection. While the tendency for natural or wild populations is to be polymorphic, i.e., to exhibit a wide range of genetic diversity, it is not established that domestic poultry need be as heterogenous.

Genetic Variation in Parasitic Invaders

The parasite, according to Sprent (1963), is any organism which can use or requires the tissues of another in part or all its life cycle. The heteroparasites are phylogenetically distinct from the host and include viruses, mycoplasmetales, rickettsiae, bacteria, molds, protozoa, worms, arthropods, etc.

trol afforded by this type of breeding and multiplication as well as the high-level performance obtained suggest that such crossing systems will persist for some time. Attention is no longer required by the breeder and flock owner to certain of the standard breed and variety defects.

There are also changes in patterns of disease incidence as poor viability stocks have been eliminated and improvement has been made in the livability of surviving strains and crosses. Some indication of this is shown in comparisons of random sample egg-laying test reports such as made by McClung (1961). It is obvious that a good deal of the competitiveness of the surviving commercial varieties is due to their superior viability.

Increased densities give greater opportunities for infective organisms to build to high concentrations and nonpathogenic organisms to become pathogenic or at least harmful secondary invaders. There seems to be an increased susceptibility to organisms normally protected against by supposedly innate resistance mechanisms. Examples of these include high early-age losses in broiler stocks from the leucosis complex, increased incidence of lymphomatosis in turkeys, and occasional epidemics of blackhead in chickens.

The increased and combined use of antibiotics may result in the evolution of new and resistant pathogenic invader types. This has been demonstrated in both penicillin and streptomycin resistance of certain microorganisms. Widespread use of antibiotics may create a risk where Salmonella and the like are a menace to man. If resistant strains are involved these agents may not respond to therapeutic use of antibiotics. Appearance of atypical forms of various common agents, such as variant forms of *Salmonella pullorum*, can always be expected to occur occasionally even without an antibiotic as a screening agent.

There has been a reduction of certain diseases through eradication methods. These include the reduction in avian tuberculosis in all pullet flocks, and eradication and prevention methods which have been effective in reducing pullorum outbreaks, although on the whole control and eradication methods have failed to eliminate the Salmonella infections. Newcastle disease in Britain did not yield to the slaughter policy. The efforts to establish PPLO-free breeding flocks have only been partially successful. Part of these failures can be blamed on not following the rules, but the biological principles involved are obviously not fully understood.

THE GENETIC MECHANISM

Chickens and other poultry are complex systems with a built-in direction for life functions. This blueprint for development and reproduction of its kind is contained in the chromosomes of the cell nucleus. Variation in the genetic direction system as well as environmental effects have a profound influence on the well-being of the organism. There seems to be a certain mysticism about genetics caused largely by the definition of a "gene" and the uncertainty of quantitative traits which seem to be only vaguely controlled by genetic mechanisms. Recent findings in cellular biochemistry and population genetics have advanced our understanding of these factors and the part they play in species preservation. It should be kept in mind that a gene has never been more than a postulate to explain an actual occurrence. It has been generally supposed that the genes or determiners were molecular in nature. Knowledge about the hereditary material suggests that there is in fact a precise molecular template. The hereditary material consists principally if not entirely of deoxyribonucleic acid (DNA) which is a long chain of four repeating nucleotides. Each nucleotide is composed of deoxyribose, phosphoric acid and either a purine or pyrimidine base. The long chain of nucleotides is believed to be one strand of the two-stranded chromosome. According to Watson and Crick (1953) the double

segregations affecting form and function, some of which are lethal or sublethal in nature. Landauer (1951) listed 17 lethals affecting embryonic development. Several more have been subsequently reported.

The rather simply inherited patho-genetic situations and genetic abnormals have been classified and discussed by both Hutt (1949) and Jull (1952). They report in some detail a considerable number affecting the skeleton, feathers, and organs. Waters and Bywaters (1959), authors of the chapter on genetics in the fourth edition of this book, also cite several examples. A number of genetically interesting and physiologically important ones have since been reported, principally in *Poultry Science* and the *Journal of Heredity*. It is entirely likely that there are many abnormalities ranging in effect from lethal to subvital which are never investigated. Hutt (1961b) has pointed out that complacency about these in poultry is ill advised as the number of the inherited genetic defects may in fact be much greater than now thought and advises vigilant breeding practice for an elimination of such defects.

The "genetic load" or proportion of subvital loci which populations carry is much greater than was once supposed, as suggested by extensive studies in various laboratory species. In most cases natural selection and/or selection by the breeder seems to have minimized the genetic load in the domestic fowl and detrimental recessives have had little effect on production performance. Sometimes in closed population such as inbred lines there is added effort to purge undesired deleterious recessives. Those with clearcut segregation are more or less automatically eliminated in the surviving inbred lines. Because of the high reproductive rate, relative cheapness, and general lack of sentiment attached to the individual chicken, extensive culling has prevented some of the devastation that has occurred in certain of the highly regarded registered cattle herds.

Any genetic mechanism which gives rise to polymorphism such as heterozygote superiority (overdominance) either per se or through close linkage of positive fitness loci with deleterious loci may discourage elimination of undesirable loci. Such loci as self-sterility allels or miotic drive and segregation distortion situations as reported in mice by Dunn (1957) and drosophila by Sandler (1962a and 1962b) are self-perpetuating.

Chromosomal Aberrations

Few of these have been recorded in poultry, largely because of the complex and obscure cytogenetic situation. However, it seems reasonable to suppose that these exist since they have been reported in drosophilia, maize, mice, and man in which there has been cytogenetic exploration. Bernier (1960) reports a 50 per cent lethality from appropriate matings which could be explained by a translocation or similar chromosomal aberration, although no cytological evidence was obtained. Newcomer (1959) described the cytological appearance of a translocation induced by X-rays in a carrier sire from the University of California flock.

In general, these sorts of genetic disturbances are not likely to be of economic importance unless there is a situation similar to that described by Dobzhansky (1955) for *Drosophila pseudoobscura* in which inversion heterozygotes were highly viable. The chromosome abnormalities were kept in the population at a high frequency even though the homozygotes were lethal. The inversions are equivalent to singly powerful loci, which substantially reduce the fitness of the population as a whole.

Phenodeviants

Lerner (1954) defined "phenotypic deviants as sporadic and ubiquitous for a given species, but which fail to exhibit clear-cut mendelian inheritance." A principal example, certainly encountered by every poultryman, is the crooked toe trait. Nutritional deficiencies, faulty incubation, wire floors, and other environmental influ-

The higher forms of parasitic organisms in the main are diploid and sexual reproduction with the usual results from recombination, segregation, independent assortment, and mutation provide for genetic variety as in poultry. However, the bacteria and viruses have vegetative reproduction and are haploid, so mutation is a major source of new genetic variation. Vegetative reproduction through duplication of the chromosome and transmission of these to each daughter cell continues identical heredity. Even though mutation of the spontaneous variety occurs only rarely (probably at a no higher rate than in more complicated organisms), the haploid condition in a rapidly reproducing organism, where one cell in suitable conditions may give rise to several million cells in less than 24 hours, provides considerable opportunity for selection to preserve new types. Recombination in bacteria has several mechanisms, conjugation, transformation, and transduction, although not of the ordinary crossing over type as in diploid organisms. The genetic flexibility of the microorganism is considerable with its potential reproductive, recombinational, and mutational abilities for meeting new environments.

Conjugation was described by Lederberg and Tatum (1946) in *E. coli* in which two cells fuse and form a cytoplasmic bridge with the chromosomes of the donor being injected into the recipient.

Transformation in bacteria was reported by Avery *et al.* (1944). DNA extracts have been made from streptomycin-resistant bacteria, and this extract placed in the growing media of streptomycin-sensitive cells. Many of the sensitive strain were converted to resistant cells and, furthermore, passed this resistance to their progeny. This permanent change in heredity is an extraordinary phenomenon of random transfer of genetic material, rather than the ordered sequence as in the usual gametic situation.

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PATHOLOGICAL CONDITIONS ASSOCIATED WITH HEREDITY

The relation of genetics to disease is undoubtedly one of the intricate and exciting aspects of pathology. Biologists are gradually unraveling the relationship of genetic framework and environmental interaction within which life processes exist. Certain of these relationships are clear as with certain of the simply inherited defects in form and function. Others are not as well understood, but show that different genomes of varieties, species, and individual members of each contain combinations of hereditary directions which are responsible for abnormals and nonviables. The pathological condition deviates from the normal and usually affects the well-being of the individual. Thus fitness is reduced so that the opportunity for maximum performance and the leaving of offspring is hindered or abolished. Those abnormal conditions which are due to mistakes in the genetic code (molecular or chromosomal) have been called congenital or innate. Many of these are single unit

segregations affecting form and function, some of which are lethal or sublethal in nature. Landauer (1951) listed 17 lethals affecting embryonic development. Several more have been subsequently reported.

The rather simply inherited patho-genetic situations and genetic abnormals have been classified and discussed by both Hutt (1949) and Jull (1952). They report in some detail a considerable number affecting the skeleton, feathers, and organs. Waters and Bywaters (1959), authors of the chapter on genetics in the fourth edition of this book, also cite several examples. A number of genetically interesting and physiologically important ones have since been reported, principally in *Poultry Science* and the *Journal of Heredity*. It is entirely likely that there are many abnormalities ranging in effect from lethal to subvital which are never investigated. Hutt (1961b) has pointed out that complacency about these in poultry is ill advised as the number of the inherited genetic defects may in fact be much greater than now thought and advises vigilant breeding practice for an elimination of such defects.

The "genetic load" or proportion of subvital loci which populations carry is much greater than was once supposed, as suggested by extensive studies in various laboratory species. In most cases natural selection and/or selection by the breeder seems to have minimized the genetic load in the domestic fowl and detrimental recessives have had little effect on production performance. Sometimes in closed population such as inbred lines there is added effort to purge undesired deleterious recessives. Those with clearcut segregation are more or less automatically eliminated in the surviving inbred lines. Because of the high reproductive rate, relative cheapness, and the general lack of sentiment attached to the individual chicken, extensive culling has prevented some of the devastation that has occurred in certain of the highly regarded registered cattle herds.

Any genetic mechanism which gives rise to polymorphism such as heterozygote superiority (overdominance) either per se or through close linkage of positive fitness loci with deleterious loci may discourage elimination of undesirable loci. Such loci as self-sterility alleles or miotic drive and segregation distortion situations as reported in mice by Dunn (1957) and drosophila by Sandler (1962a and 1962b) are self-perpetuating.

Chromosomal Aberrations

Few of these have been recorded in poultry, largely because of the complex and obscure cytogenetic situation. However, it seems reasonable to suppose that these exist since they have been reported in drosophila, maize, mice, and man in which there has been cytogenetic exploration. Bernier (1960) reports a 50 per cent lethality from appropriate matings which could be explained by a translocation or similar chromosomal aberration, although no cytological evidence was obtained. Newcomer (1959) described the cytological appearance of a translocation induced by X-rays in a carrier sire from the University of California flock.

In general, these sorts of genetic disturbances are not likely to be of economic importance unless there is a situation similar to that described by Dobzhansky (1955) for *Drosophila pseudoobscura* in which inversion heterozygotes were highly viable. The chromosome abnormalities were kept in the population at a high frequency even though the homozygotes were lethal. The inversions are equivalent to singly powerful loci, which substantially reduce the fitness of the population as a whole.

Phenodeviants

Lerner (1954) defined "phenotypic deviants as sporadic and ubiquitous for a given species, but which fail to exhibit clear-cut mendelian inheritance." A principal example, certainly encountered by every poultryman, is the crooked toe trait. Nutritional deficiencies, faulty incubation, wire floors, and other environmental influ-

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thesis. The Australorps were either unable to carry out the synthesis or else did so at an inefficient level. Hess et al. (1962) were able to differentiate lines into high and low methionine requirement through selective breeding. McDonald and Beilharz (1962) reported differences in calcium metabolism between strains of White Leghorns and Australorps which lead to a genetic susceptibility to low calcium rickets in the Australorps. Differences of genetic origin have been reported for utilization of vitamin D by Lillie and Bird (1949), and of vitamin E by Howes and Hutt (1952). Genetic differences in arginine requirement among White Leghorns were reported by Nesheim and Hutt (1962).

Kondra and Hodgson (1961) reported genetic differences in energy-protein requirements of chickens, as did Siegel and Wisman (1962). Nordskog and Johnson (1953) found a significant breed-antibiotic level interaction for growth to eight weeks of age. Arroyave et al. (1957) found genetic differences between and within breeds in nutrient content of eggs. McNary and Bell (1957) reported a reciprocal maternal effect in which the egg contained either a growth depressant or a nutritional deficiency.

Stutts et al. (1957) and Wilcox et al. (1962) found genetic differences in levels of a blood enzyme, serum alkaline phosphatase, which were related to egg production. The latter authors were able to select differentially for high and low lines. Glutathione concentration had a genetic correlation to level of egg production according to Stutts et al. (1956). All of the cited investigations are either directly or indirectly implicated in pathological conditions and most certainly are concerned with the viability of the bird under domestic conditions.

Immunogenetics

There has been considerable investigation in the fowl regarding the immuno-genetic relationship of the antigen-anti-

body reaction of red blood cells, the homograft transplantation reaction and the graft-against-host reaction. The investigations of Schierman and Nordskog (1961 and 1963) have shown that the B blood group locus is a major histocompatibility locus. The skin-graft and graft-against-host reactions are intimately related genetically to this locus in the chicken. Since these or similar types of reactions are involved in defense mechanisms and in some way seem to have importance in survival value it seems appropriate to discuss them at this point. No antigenic reaction has been described such as the human Rh complex which leads directly to a pathological condition.

Blood Grouping

Some six loci for red cell antigens have been identified through agglutinating iso-immune sera in the domestic fowl. Genetic tests indicate that each are located at different chromosomal regions. Presumably these loci are on different or nonhomologous chromosomes.

Certain combinations of blood group loci, and the B group in particular, confer or are associated with viability either during embryonic, growing, or adult stages of the chicken's life. There is evidence that the heterozygous condition has superiority to one or both homozygotes in the majority of cases studied: Briles et al. (1950), Schultz and Briles (1953), Briles et al. (1953), Gilmour (1954), Briles and Krueger (1955), Briles (1956a, b, and c), Briles et al. (1957), Gilmour (1959), Gilmour (1960a and b), Allen (1960), Fanguy et al. (1961), and Bumgardner et al. (1961). These findings suggest that the loci for chicken red cell antigens are in a state of balanced polymorphism, i.e., enforced heterozygosity, because of general fitness superiority. In any case there is no clue so far as to how or why the identified loci confer the apparent fitness. Certain of the theoretical implications and applied breeding aspects are discussed by Briles (1956b and c) and Gilmour (1960b and 1962).

ences affect the appearance of this abnormality. Certain strains have been known to show this defect more than others in indicating a feasible genetic influence. Hicks and Lerner (1949) found that certain inbred lines had a high incidence. Also incidence could be increased and decreased through selection. However, there seem to be few if any cases of simple segregation ratios found in breeding tests for crooked toes in the domestic fowl.

The anomaly of crooked keels which has plagued poultrymen for years likely falls into a similar category of a character determined by and conditioned in its expression by known environmental situations, such as roosts and vitamin D, with a high incidence in certain strains and lines. The genetic situation as reported by Warren (1937), Waters (1949), Shoffner et al. (1953), Hyre (1955), and Shoffner and Canfield (1957) shows that incidence may vary greatly depending upon genetic background.

The self-dubbing trait reported by Bernier (1961), the baldspot and cleft-palate traits by Shoffner et al. (1953), and the multiple side sprig condition by Taylor (1946) all have obvious phenotypic expression but ill-defined genetic influences. The fact that these traits appear more or less unexpectedly and have been observed in many flocks of chickens suggests that these, and other characters with similar action, are conditioned by numerous loci, and when a certain fraction becomes homozygous the trait is expressed. In a fairly large interbreeding flock the trait appears only occasionally, but if there is concentration of ancestry either through selection or deliberate inbreeding, homozygosity can increase the incidence.

PHYSIO-BIOCHEMICAL GENETIC VARIATIONS

Dietary and Metabolic

Some of the better known mutant forms in molds and bacteria which are simply inherited are concerned with nutritional capacities and synthesis from crude products to the more refined constituents needed in metabolism. It is well known that nutritional deficiencies in poultry not only produce specific pathological conditions, but can also contribute to susceptibility or resistance to invading parasites. In general, attempts to modify diets through superabundance of vitamins, amino acids, or growth factors in an attempt to increase resistance have been ineffectual according to Hill (1962). Therapeutic levels of antibiotics are obvious exceptions.

Nearly everyone who has studied the question of existing differences in dietary requirements, utilization, or metabolism of poultry, has found that families, lines, strains, and breeds do show differences Unfortunately, in most cases our knowledge goes little beyond this demonstration. Fortunately it does emphasize that such discrepancies do exist, and such investigations will contribute to our knowledge of the biology of the fowl. Just when extensive investigation of these inherited differences will lead to an applied breeding program for increased fitness and performance is difficult to diagnose.

Hutt (1949, 1958) has reviewed the literature relating to genetic differences in diet utilization in chickens. Genetic differences have been demonstrated for thiamine requirement by Howes and Hutt (1956), Thornton (1960), Lamoreux and Hutt (1939), and Roberts (1962).

Differences in inherited capacity to utilize riboflavin was shown by Lamoreux and Hutt (1948). Maw (1954) reported a recessive single gene mutant involving the metabolism of riboflavin. The homozygous adult female is unable to transfer sufficient riboflavin from the diet to the egg for embryonic growth and development. Cowan et al. (1961) have shown that the normal laying hen retained free riboflavin more effectively than the laying hen which was homozygous recessive for this trait.

McDonald (1957, 1958) found breed differences between White Leghorns and Australorps in methionine and cystine syn-

secondary in occurrence rather than a primary causative factor. Kondra and Cavers (1947) found that the incidence of keel cysts was intimately related to the rate of feathering, particularly in the region of the keel, and concluded that the appearance was largely a defense mechanism. Gyles et al. (1959), Shoffner and Canfield (1957), and Hyre (1955) have presented evidence that the presence of keel cysts and other deformities of this region are influenced to some degree by heredity. Gyles et al. (1962) differentially selected for high and low incidence lines in a heavy broiler strain. They found the heritability for incidence of the blisters to be between 16 and 24 per cent in the high incidence line and zero in the nonblister line.

Climatic

Hutt (1938), Kheireldein and Schaffner (1957), and Huston et al. (1957) have variously demonstrated that there are genetic differences in susceptibility to controlled high temperatures. These temperatures were extreme, acting as a "stressor" to the point where some individuals would succumb. Campos et al. (1960) showed that there were breed differences in performance responses to fast and slow rises in ambient temperature. Siegel and Mueller (1955) reported that outbreds and crossbreds had greater resistance than inbreds to exposure to low temperature.

A very interesting contrast to extreme external environment was the result reported by Greenwood (1958) on the longterm effects of a constant environment. Birds maintained in a climatic chamber with constant environment did not perform as well as controls influenced by naturally fluctuating climatic conditions. Both Greenwood (1958) and Wilson (1958) reported that the constant environment resulted in almost complete incidence of adenocarcinomata involving tumors of ovaries and oviducts.

Feeding Practices

A currently widespread commercial practice is to restrict intake of feed for replacement pullets for both broiler breeder females and egg production stocks. In general the restriction practice delays sexual maturity and shifts the egg laying pattern and usually improves laying house livability. The publications of Hollands and Gowe (1961) and Gowe et al. (1960 and 1962), in which careful and extensive investigation of response of different genotypes were observed for both full and restricted feeding, reported that the restricted birds had higher livability during the production year. Different strains, though varying among themselves in viability, tended to maintain their rank in either environmental treatment.

Biely and March (1959) placed strains of White Leghorns, both resistant and susceptible to the avian leucosis complex, on high and low planes of nutrition. All strains on the high plane of nutrition had a greater incidence of avian leucosis complex. Waters et al. (1950) found in certain inbred lines of White Leghorns developed for resistance and susceptibility to the avian leucosis complex that there were genetic differences in livability for the first 21 days after hatching. Modification in the starting diet markedly altered the mortality pattern.

Location Effects

Experimental and commercial strains have been compared under a variety of environmental conditions, including such comparisons as cage vs. floor, farm to farm, one random sample egg laying test to another, and time of year to time of year. While management practices of comparisons vary, a not surprising suggestion is that infectious agents of one kind or another are involved in the genotype-environmental interaction observed. That is, the discrepancies between levels of performance in the different locations are due to morbidity and/or mortality from uncontrolled and unrecognized infections. The essence of this problem resolves to whether the top genotypes consistently do better under various environments or if there are significant interactions; if so,

Graft-Against-Host Reaction

This is a reaction of an immunologically competent graft against the foreign antigens of the host. In the cases cited here the host is a chicken embryo, presumably immunologically incapable of producing antibodies. Burnet and Burnet (1960 and 1961) found clear genetic segregation for graft-against-host reaction of adult leucocytes on the chorioallantoic membrane of the chick. Jaffee and Payne (1962) presented evidence for inheritance of differences of the graft-against-host reaction between two inbred lines of chickens which differed in at least one major locus determining antigens responsible for the splenomegaly reaction in the embryo.

Host-Against-Graft Reaction

This sort of reaction commonly occurs when grafts of tissues and organs are made from one individual to another. When antigens of host and donor are the same (twins and members of isogenic lines) the reaction does not occur. Craig and Hirsch (1957), Craig et al. (1960), Berry and Craig (1959), and Polley et al. (1960) have shown that there are genetic differences in skin graft histocompatibility reactions of chickens.

GENETIC-ENVIRONMENTAL INTERACTIONS

The experienced biologist recognizes from long experience that a variety of environmental influences may "stress" an organism to the point of disorganization. Development of specific management practices in intensification has led to investigation as to whether there are definite and repeatable genetic effects and whether strains perform differently in various environments.

Social Order

The hierarchical social system or peck order in the domestic fowl is intimately related to temperament and husbandry practices. The publications of Guhl (1953 and 1962) have summarized the investigations which have shown rather clearly the relationship between social rank and productivity. The ones on the low end of the social scale are usually below normal in their physical state and sometimes are at the point of starvation. McBride (1958, 1960, and 1962) presents evidence to support the hypothesis that the curvilinear relation of productivity and rank depends to a large extent on availability of feed, water, bird density, and other management factors.

Behavior per se is not inherited but certain genotypes have different physiological responses to stimuli. Certain strains of chickens dominate others in mixed groups. Temperament is not presently definable in simple genetic terms, except that certain strains are reproducibly and predictably dominant or subservient. Guhl (1953), Tindell and Craig (1959 and 1960), and Guhl et al. (1960) have demonstrated that genetic variation in social aggressiveness and competition effects exists within strains, between sire families, strains, inbred lines, and crosses. Selection for high and low aggressiveness showed considerable response.

Dickerson et al. (1961) investigated the heritability for picking behavior in chickens and found it to be low. The measurable heritable differences were among sire and dam families for aggressiveness. Whether social aggressiveness is intimately tied in with such vices as feather picking, vent picking-prolapse, or other cannibalistic habits is difficult to ascertain as prolapse is presumed to be physiologically unrelated. Practical experience has shown that some strains of birds are more prone to these tendencies than others. There has been discrimination by the producer in recent times against strains with prolapse tendencies.

Breast Blisters

The incidence and size of keel cysts on growing chickens and turkeys depends upon inherited tendency, type of floor, moisture in the litter, and micro-invaders, although it would seem that the latter are

little or no selection progress due to the low heritability of viability.

Resistance measurement usually is a discontinuous variable: either the individual has a pathological condition or it has not. However, morbidity may be graded into classes of low, medium, and high. In cases where resistance or nonresistance is measured by death, the trait becomes all-or-none, and groups are divided into those that die and those that survive. It is generally assumed that the genotypes form a continuous scale for resistance, but because of the threshold manifestation, phenotype is a discontinuous variable. As a result the geneticist often converts to some scaling method such as probits which are amenable to statistical manipulation. Certain of the pertinent considerations and useful genetic techniques for viability selection are discussed by Lerner (1958) and Falconer (1960).

CONSIDERATIONS IN RECOGNIZING RESISTANCE

With few exceptions, the investigations of resistance in poultry have explained little about pathways and mechanisms involved for either general or specific manifestation of resistance on the part of the host to pathogenic organisms. Mention is made here of certain of the recognized protective mechanisms involved in resistance as a reminder that several pathways and physiologic reactions may be involved and to focus attention on the precautions necessary in a resistance recognition program. Knowledge of principles involved in immunity and resistance are more valuable than merely recognizing resistance. The answer to whether resistance is through some barrier excluding entry of the organism or is through ability to harbor the agent and not succumb will permit a more knowledgeable approach to a selection program or any plan for protection against the pathogen. The possibility of using physio-chemical tests to predict biological fitness is attractive to reduce cost and increase accuracy. A perfect correlation between a biophysical measurement and viability does not increase the heritability of the viability trait. It may, however, provide an early, inexpensive, and accurate estimate of final performance. A physiological or biochemical measurement in a parent may predict the viability of progeny to a higher degree than does the viability of the parent.

Kinds of Immunity

In innate immunity the internal or external environment of the host is entirely unsuitable for the invader, and includes such things as age, body temperature, and anatomical and physiological differences. Frequently innate means we do not know why one organism is not readily infected by another, it just is not.

Natural immunity mechanisms are of a considerable variety and include the following: entry surfaces which are barriers to infection by secretion of such antiparasitical substances as lysozyme, fatty acids, high and low pH, etc.; antiparasitical substances in the blood or other body fluids which include antibodies, antienzymes, lactic acid, blood complement, and gamma globulins; and the phagocytic system which is a universal and fundamental mechanism in resistance. There are individual differences in ability to form antibodies and in ability to ride out an infection until time has been gained for manufacture of antibodies.

Acquired immunity is not necessarily a characteristic of the species but varies according to the history of infection of the individual, much like protective immunization. Passive immunity such as that passed through the egg to the chick is not lasting, and others of this sort may very well obscure natural abilities for resistance.

Hypersensitivity or allergic reactions are frequently associated with worm and arthropod infection and may be of considerable significance in preventing the penetration of metazoan parasites.

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Ecological immunity occurs when the characteristic behavior and feeding habits

are such that neither nonexposure nor non-infection can take place.

CONSIDERATIONS IN DETECTING RESISTANCE

Specificity

The specificity of resistance is due largely if not entirely to the specificity of antigen-antibody reaction. The synthesis of a specific globulin involves the production of a specific pattern to the antibody molecule which renders the antibody capable of reacting only with the corresponding or closely related antigen. Likewise, genotypic resistance to a particular organism is specific as continually found by those involved in their immunological procedures and selective efforts to increase resistance.

Examples of specific resistance are shown in the findings of Cole (1941), Carson (1951), and Patterson et al. (1961) where stocks resistant to one infective agent were not necessarily resistant when exposed to other agents. This is convincing evidence that selection for specific resistance rarely results in over-all resistance. Waters and Burmester (1963) emphasize the fact that a specific strain RPL 12 virus and specific isolate genotypes (inbred lines) were instrumental in pinpointing the mode of inheritance to erythroblastosis.

Etiology

The fact that resistance exists and can be increased by selection without a complete knowledge of the etiology, as for example that for the avian leucosis complex, does not minimize the importance of knowing the etiology. Chronic respiratory disease with multiple etiology, according to Chu (1962), is another example of the complexity of a disease. If one organism gives multiple or variable syndromes or if several organisms give a similar clinical syndrome, there will be uncertainty about control measures until cause and effect are determined.

Exposure

Obviously unless and until individuals are exposed there is no way of recognizing their ability to resist an infectious agent, except for a predetermined relationship with some other physiological trait. One alternative is to use collateral family information, i.e., expose a portion of a stock and use nonexposed full sibs, half sibs, or progeny-tested parents for the production of progeny. This bypasses reproductive and dissemination problems from exposure but in no way lowers the requirements of exposure technique in the portion of the population tested. At least half of the genetic variance information contributing to selection progress is lost in going from the individual to information from relatives. The opportunity for mistakes in selection is obvious when only a part of the population is exposed. Unfortunately conditions of natural exposure often do not completely expose the entire population. Occasionally, natural infections may go undetected as in some respiratory diseases where infection was not suspected until subsequent tests disclosed high titers.

The relationship between responses to natural and artificial exposure and whether or not such responses are controlled by the same genetic factors are considerations in ensuring adequate exposure and constant selection pressure. Tests of correlation between inoculation, Champion (1954), sporulated oocysts; Heisdorf et al. (1947), with oral dosage; and Burmester et al. (1953), with cell-free extracts and natural exposure were reasonably satisfactory. Goodwin's (1957) description of the method and result of proximity brooding of chicks to carrier hens (leucosis complex) in an effort to ensure equal and adequate exposure suggests that this method is not entirely satisfactory. The numerous and uncontrollable factors such as transfer of passive immunity to progeny by parents with latent infection (Burmester, 1955 and Burmester et al., 1957) are a continual threat to evaluation of resistance.

Exposure to infective agents under natural conditions is sporadic so that occasionally the survivors of infection may not have their progeny rechallenged by the same agent for many generations. Mean-

while natural selection may be assorting the genotype in another direction. Under artificial selection conditions, however, persistant and renewed pressures are the ones that will establish new and improved levels of resistance which becomes a part of fitness.

MODES OF RESISTANCE IN THE FOWL

Certain of the phenomena and manifestations mentioned in this section are clearly associated with differential viability. However, it should not be construed that these are in themselves either cause or effect but are likely stages or states in which the physiological response can be recognized.

Heterosis

The phenomenon of increased vigor on crossing is well recognized in both plants and animals and, in general, the more isolated or inbred the populations, the greater will be the performance of the cross over the parent stocks. Embryo viability is almost universally increased as the result of crossing according to Warren (1942), Hutt (1919), Warren (1958), and especially if the female parent is a cross according to Shoffner (1948). However, King and Bruckner (1952) found little if any increase in hatchability of strain and breed crosses over parent stocks. As a general rule heterotic viability effects are pronounced during the brooding and rearing period as indicated by Warren (1942), Goodwin et al. (1956), and Hutt (1919). Siegel and Mueller (1955) found that outbred and crossbred chicks had a greater relative resistance to a low temperature exposure than did the chicks from either parental stock.

A proportion of experimental crosses shows an increase in adult or laying house viability. Goodwin et al. (1956) reported that strain-cross progeny were superior in pure-strain progeny in ability to withstand certain respiratory infections in layinghouse adults. Others, such as King and Bruckner (1952), found no difference between parent stocks and their crosses in total mortality and occurrence of mortality

due to neoplasms. The expectation for increased adult viability in the cross has been notably violated in the reduction of mortality from the leucosis complex. Hutt and Cole (1952) reported that the cross progeny of two White Leghorn strains relatively resistant to leucosis had slightly higher rate of death from neoplasms than progeny of either parent stock. Waters (1951b) using a series of crosses between inbred lines which varied in degree of resistance to avian lymphomatosis found no decrease in mortality from the inbred parent stocks. Bearse et al. (1961 and 1963) reported that leucosis mortality was not altered by the crossing of selected resistant and susceptible lines although there was lowered mortality from other causes. These experiences suggest that heterotic vigor per se will not automatically confer increased resistance to the avian leucosis complex. This unpredictability or deviation from that expected undoubtedly has several possible explanations. One is that the hybrid provides a "better" environment for agents inducing the malignancy.

Reciprocal Effects

Warren (1912) brought attention to the fact that there was a noticeable difference in performance between reciprocal crosses of Leghorns and heavy breeds. Reciprocal differences for sexual maturity and broodiness had been noted in several previous investigations and confirmed in Warren's excellent study. Warren noted that when White Leghorn males were mated to Rhode Island Red females, the mortality of the progeny was higher than that from the reciprocal cross. Because of the error involved in the estimation of mortality, it was not immediately apparent that this phenomenon was a rather general rule. However, subsequent reports such as Warren and Moore (1956), Hutt (1961a), and others leave little doubt that differences between reciprocal crosses are real.

Nordskog and Phillips (1960) presented evidence to show that progeny of the White Leghorn male of a Leghorn heavy cross have higher mortality than the reciprocal

and suggested that the Leghorn sex chromosomes are in some way associated with adult mortality. Moultrie et al. (1953) crossed strains of White Leghorn and secured reciprocal differences and implied that maternal effects may account for the difference.

Allen (1962) attempted to distinguish the contribution of sex linkage and maternal influence in the Leghorn-heavy cross. He found as usual that the Leghorn male × Rhode Island Red female produced progeny with significantly lower adult viability than the reciprocal cross. A series of especially designed backcrosses were made to study the effect of extranuclear (maternal) influence. A "plasmon" effect was noted which was interpreted as an interaction of the sex chromosome of White Leghorn and extranuclear influence of the Rhode Island Red.

The progeny from a heavy male and Leghorn female not only have higher viability but also have a higher incidence of broodiness. While some speculation has been made regarding the association of broodiness and increased viability, the phenotypic correlation may be fortuitous as the same genes may or may not be involved nor even have common physiological relationships. The possible sex linked inheritance, maternal effects (both cytoplasmic and viral agents), and interrelationships of hormonal secretion may influence the differential response observed in this type of reciprocal cross.

Bursa of Fabricius

The bursa of Fabricius is the primary source (activator) and the spleen is inactive or secondary in the production of antibodies in the young chick. The researches of Jaap (1958 and 1960), Chang et al. (1955 and 1957), Oakberg and Lucas (1949), Oakberg (1951), Glick (1955 and 1960), and Sadler and Glick (1962) have demonstrated heritable differences in both size and antibody production. The size of the bursa apparently has a relationship with the quantity of antibody produced. The ob-

served higher viability of some strains seems associated with large bursa size. Just how this supposedly temporary mechanism fits into genetic resistance is not yet clearly defined.

Body Temperature

One of the innate mechanisms which may differentiate resistance to a specific organism from one animal to another is body temperature. Hutt (1955) and Lamoreux and Hutt (1939) found that White Leghorn chicks were able to raise their body temperatures more quickly than Rhode Island Reds. This supplied a lead to a possible physiological basis of resistance to Salmonella pullorum. Considerable investigation was reported at various times by Hutt and Scholes (1911), Ram and Hutt (1955), and Hutt (1958) in which the genetic resistance of chicks to Salmonella pullorum was associated with the ability to accelerate the transition from the poikilothermic state of the embryo to that of the homoiothermic during the first 10 days after hatching. The rise in total leucocytes in response to infection seemed not to be genetically differentiated. Hutt and Crawford (1960) found that selection in both Rhode Island Reds and New Hampshires for high and low body temperature was effective in differentiating lines after two cycles of selection.

Sex

The balance of sex hormones may influence the incidence of lymphomatosis as Burmester (1945) and Burmester and Nelson (1945) observed nearly double the incidence in females as compared to that of males. The influence of castration and sex hormones on incidence was studied and the lack of male hormone was implicated in the increased incidence of lymphomatosis found in females. The incidence of keel cysts is higher in males, largely because the smaller females feather somewhat faster over the keel area according to Kondra and Cavers (1947). Of course those pathological conditions associated with egg

laying such as cystic ovaries, ruptures, and prolapses are sex limited.

Age

The embryonic chick and newly hatched chick are immunologically incompetent. The susceptibility to coccidiosis decreases with age. Hutt *et al.* (1944) and Waters and Bywaters (1949) showed that early exposure of chicks to lymphomatosis will lead to much higher incidence of adult mortality from that cause than in chicks exposed later in life. Their findings lead to the well-known management recommendation of chick isolation from older birds which works so well.

EXPERIMENTAL

Inbred lines and other selected populations have identifiable and reproducible genotypes which should not be overlooked by the investigator as a means of comparison and identification in biological assay. The genetic variability, along with environmental sources of variance, influence assay methods for the estimation of the potency of drugs, antibiotics, vaccines, bacterins, virulence of microorganisms, isolation and identification of agents, resistance, and susceptibility. The variation between individuals may be so large that judgments from one or few are misleading; however, averages of a group are usually reliable for distinguishing differences in capacity.

Some of the selected and isolate genotype stocks which have facilitated certain prior investigation and should be invaluable for contemplated research are the following: the RPL inbred lines which are resistant and susceptible to leucosis according to Waters and Burmester (1963); strains resistant and susceptible to leucosis according to Hutt and Cole (1948); lines with differential body temperature according to Hutt and Crawford (1960); lines with increased bursa of Fabricius sire according to Jaap (1960); stocks with single gene segregates, stemming principally from highly inbred stocks, as blood group loci according to Briles (1956b); the Rous sarcoma and erythroblastosis loci according to Waters and Burmester (1963); and the riboflavin deficiency locus according to Maw (1954).

On the other hand, inbred or heavily selected stocks, even though relatively homogeneous genetically, may be more subject to extraneous environmental influences since they are frequently less adaptive than heterozygous stocks. Lerner (1954 and 1958) discusses the topic of genetic buffering in some detail and suggests that under adverse environmental conditions the heterozygote will be less variable than the homozygote. When testing for a universal hypothesis or application the use of several genetic sources of either outbred, randombred, or crosses in conjunction with different treatment levels will give results of a more reliable nature in that estimates for the relative magnitude of genetic, treatment, and interaction effects are obtained.

The clinician, diagnostician, practitioner, pathologist, and others occasionally have encountered unexpected and unexplained variations which have been laid to genetic causes. Some caution should be exercised, however, as other sources of variation may be involved in the aberrants observed. For instance, field observations oftentimes point to strain or breed specificities but more often than not a particular strain is associated with a particular environment whose peculiarities have distinct influences.

The adaptivity of the fowl is continually challenged because of changes in management practices. The quantitative variation in the several genotypes for resistance as previously described, suggest that many loci with different effects are concerned in their inheritance. Furthermore the complicating influence of other environments serves to obscure the heritability of any specific one. Consequently, the staggering investment in selective breeding for specific invader resistance as well as adaptation to climatic, nutritional, or intensivism extremes may be viewed by some as question

able. Especially this is true since the breeder is often unable to predict just what stress someone may impose on his stock, or whether there are enough potential customers for a special stock to justify the investment. Numerous unpredictable and peculiar notions by the poultry industry about certain genetically influenced traits have occurred in recent years. It has variously favored or discriminated against comb type, eggshell color, plumage color, and skin color so that these seemingly inoffensive characteristics became defectives.

REFERENCES

Ablanalp, H., Marrou, L. F., and Goto, F.: 1962 Genotype-environment interaction in laying tests of poultry. Poultry Sci. 41:927.

Ackert, J. E., Eisenbrandt, L. L., Wilmoth, J. H., Glading, B., and Pratt, I.: 1935. Comparative resistance of five breeds of chickens to the nematode *Ascaridia lineata*. Jour. Agr. Res. 50 607.

Allen, C. P.: 1960. A specific isoimmune chicken antiserum which identifies A locus alleles and B locus heterozygotes within Leghorn lines Genetics 45:971.

———: 1962. The contribution of the plasmon to specific reciprocal cross differences in poultry. Poultry Sci. 41:825

Arroyave, G., Scrimshaw, N. S., and Tandon, O. B.: 1957. The nutrient content of the egg of five breeds of hens. Poultry Sci. 36:469.

Asmundson, V. S., and Biely, J.: 1932. Inheritance of resistance to fowl paralysis (*Neurolymphomatosis gallinarum*). I. Differences in susceptibility. Canad. Jour. Res. 6:171.

Avery, O. T., Macleod, C. M., and McCarty, M.: 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Jour. Exper. Med. 79:137.

Beadle, G. W., and Tatum, E. L.: 1941. Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. 27.499.

Beard, J. W.: 1956. Virus of avian myeloblastic leukosis. Poultry Sci. 35:205.

Beard, J. W., Eckert, E. A., Sharp, D. G., and Beard, D. W.: 1955. Virus of myeloblastosis, a leukemie form of avian leukosis. Poultry Sci 31:1179.

Bearse, G. E., Becker, W. A., and Hamilton, C. M.: 1961. Resistance and susceptibility to the leukosis complex in chickens. Poultry Sci. 40:1377.

———, Becker, W. A., and Hamilton, C. M.: 1963. Resistance and susceptibility to the avian leukosis complex in chickens. Poultry Sci. 42:210.

———, McClary, C. F., and Miller, M. W.: 1939. The results of eight years of selection for disease resistance and susceptibility in White Leghorns. Poultry Sci. 18:400.

Bell, A. E.: 1949 Physiological factors associated with genetic resistance to fowl typhoid. Jour. Infect. Diseases 85:184.

Bernier, P.: 1960. A spontaneous chromosome aberration in a S.C.W. Leghorn. Poultry Sci. 39 1234

———: 1961. Self-dubbing: a phenodeviant in single comb White Leghorns. Poultry Sci. 40.1378.

Berry, J. E., and Craig, J V: 1959. Estimation of genetic diversity among strains and breeds of chickens by lymphocytic increases following skin grafting Poultry Sci. 38,439.

Biely, J., and March, B. E.: 1958 Strain differences in susceptibility of chickens to renal disorders. Poultry Sci. 37:990.

———, and March, B. E.: 1959. Genetic and nutritional effects on the incidence of the avian leucosis complex. Poultry Sci. 38:1101.

———, Palmer, L., Lerner, I. M., and Asmundson, V. S.: 1933. Inheritance of resistance to fowl paralysis; *Neurolymphomatosis gallinarum*. Science N. S 78:42.

Briles, W. E.: 1956a The relationship between B blood group genotypes and adult performance in two White Leghorn inbred lines. Poultry Sci. 35.1134.

———: 1956b. Individual blood group differences for blood group genes 5th Poultry Breeders' Roundtable. 32–53 DeKalb Agr. Assn. Inc a. Sycamore, Ill.

———: 1956c. Superiority of birds heterozygous for blood group genes 5th Poultry Breeders' Roundtable 78–100.

———, Allen, C. P., and Millen, T. W.: 1957. The B blood group system of chickens. The B blood group system in closed populations. Genetics 42:631.

———, Johnson, L. W., and Carger, M. J.: 1953 The effect of heterozygosity at the blood group locus B on weight at nine weeks of age in related inbred lines of White Leghorns Poultry Sci. 32:890.

———, and Krueger, W. F.: 1955 The effect of parental B blood group genotypes on hatchability and liveability in Leghorn inbred lines. Poultry Sci. 34:1182

———, McGibbon, W. H., and Irwin, M. R.: 1950. On multiple alleles affecting cellular antigens in the chicken. Genetics 35.633.

Bumgardner, H. L., Blow, W. L., Garren, H. W., and Murphy, J. W.: 1961. A study of the A and B blood type systems and mortality from fowl typhoid. Poultry Sci. 40.1384.

Burmester, B. R.: 1945. The incidence of lymphomatosis among male and female chickens. Poultry Sci. 24:469.

————: 1955. Immunity to visceral lymphomatosis in chicks following injection of virus into dam. Proc. Soc. Exper. Biol. Med. 88:153.

————: 1956. The shedding of the virus of visceral lymphomatosis in the saliva and feces of individual normal and lymphomatosis chickens. Poultry Sci. 35:1089.

————, and Gentry, R. F.: 1954. The transmission of avian visceral lymphomatosis by contact. Cancer Research 14:34.

————, and Nelson, N. M.: 1945. The effect of castration and sex hormones upon the incidence of lymphomatosis in chickens. Poultry Sci. 24:509

————, Walter, W. G., and Fontes, A. K.: 1957. The immunological response of chickens after treatment with several vaccines of visceral lymphomatosis. Poultry Sci. 36:79.

————, Waters, N. F., and Gentry, R. F.: 1953. The relationship of the response of families of chickens to natural exposure and to inoculation with the agent of visceral lymphomatosis. Poultry Sci. 32:890.

————, and Waters, N. F.: 1956. Variation in the presence of the virus of visceral lymphomatosis in the eggs of the same hen. Poultry Sci. 35:939.

Burnet, D., and Burnet, F. M.: 1961. Analysis of major histocompatibility factors in a stock of closely inbred White Leghorn fowls using a graft versus host reaction on the chorio-allantoic membrane. Australian Jour. of Exper. Biol. and Med. Sci. 39-101.

Burnet, F. M., and Burnet, D: 1960. Graft versus host reactions on the chorio-allantoic membrane of the chick embryo. Nature 188:376.

Campos, A. C., Wilcox, F. H., and Schaffner, C. S: 1960. The influence of fast and slow rises in ambient temperature on production traits and mortality of laying pullets. Poultry Sci. 39:119.

Carson, J. R.: 1951. Exposure to disease agents of strains of chickens differing in resistance to leucosis. Poultry Sci 30:213.

Champion, L. R.: 1954. The inheritance of resistance to cecal coccidiosis in the domestic fowl. Poultry Sci. 33:670.

Chang, T. S., Glick, B., and Winter, A. R: 1955. The significance of the bursa of Fabricius of chickens to antibody production. Poultry Sci. 34:1187.

————, Rheins, M. S., and Winter, A. R: 1957. The significance of the bursa of Fabricius in antibody production in chickens. 1. Age of Chicken. Poultry Sci. 36:735.

Chu, H. P.: 1962. The multiple aetiology of chronic respiratory disease complex. Proc. Twelfth World's Poultry Cong. Symposium Papers, Sydney, Australia.

Cole, R. K.: 1941. Genetic resistance to a transmissible sarcoma in the fowl. Cancer Research 1:714.

————: 1950 Differences in familial incidence of mortality from "Blue Comb" disease. Poultry Sci. 29:598.

————, and Hutt, F. B.: 1961. Genetic differences in resistance to Newcastle disease Avian Dis. 5:205.

Coles, R.: 1955. Some observations on breeding fowl resistant to lymphomatosis. Poultry Sci. 34:312.

Cowan, J. W., Buss, E. G., and Boucher, R. V.: 1961. Physiological characteristics associated with a mutant gene in chickens that cause a deficiency of riboflavin. 3. Total excreta and and urine. Poultry Sci. 40:1590.

Craig, J. V., and Hirsch, L. J.: 1957. Genetic relationship and the reaction to skin grafts. Jour Heredity 48:235.

————, Polley, C. R., and Wearden, S.: 1960. Estimation of genetic diversity by skin-graft reactions in young chicks. Poultry Sci. 39:1533.

DeVolt, H. M., Quigley, G. D., and Byerly, T. C.: 1941. Studies of resistance to pullorum disease in chickens. Poultry Sci. 20:339.

Dickerson, G. E.: 1960. Genetic environmental interaction in field testing of egg strain chickens. Poultry Sci. 39:1244

————, Kashyap, and Lamoreux, W. F.: 1961. Heritable variation in picking behavior of chickens. Poultry Sci. 40:1594.

Dobzhansky, Th.: 1955. A review of some fundamental concepts and problems of population genetics. Cold Spring Harbor Symp. Quant. Biol. 20:1.

Dunn, L. C.: 1957. Evidence of evolutionary forces leading to the spread of lethal genes in wild population of house mice. Proc. Nat. Acad. Sci 43-158.

Eckert, E. A., Beard, D., and Beard, J. W.: 1956. Virus of avian erythroblastosis 1. Titration of infectivity. Jour. Nat. Cancer Inst. 16.1099.

Edgar, S. A., King, D. F., and Johnson, L. W.: 1951. Control of avian coccidiosis through breeding or immunization. Poultry Sci. 30 991.

Falconer, D. S.: 1960. Introduction to Quantitative Genetics Oliver & Boyd, Edinburgh and London.

Fanguy, R. C., Ferguson, T. M., and Quisenberry, J. H.: 1961. The effect of blood groups upon hatchability and adult livability Poultry Sci. 40:1400.

Francis, D. W., and Kish, A. F.: 1955 Familial resistance to Newcastle disease in strain of New Hampshire. Poultry Sci. 34:331.

Gentry, R. F., and Burmester, B. R.: 1955 Tumor incidence in the progeny of hens repeatedly injected as adults with visceral lymphomatosis virus. Poultry Sci. 34:41.

Gildow, E. M., Williams, J. K., and Lampman, C. E.: 1940 The transmission of and resistance to fowl paralysis (lymphomatosis). Idaho Agr. Exper. Sta. Bul. 235.

Gilmour, D. G.: 1954. Selective advantage of heterozygosis for blood-group genes among inbred chickens. Heredity 8:291.

———. 1959. Segregation of genes determining red cell antigens at high levels of inbreeding in chickens Genetics 44 14.

———. 1960a. Blood groups in chickens a review. Rept. VI Int. Blood Group Cong.: 50–79. Inst. fur Blutgruppenforschung, Tierzuchtforschung e v Munich.

———. 1960b. Blood groups in chickens. Brit. Poultry Sci. 1:75.

———. 1962. Current status of blood groups in chickens. Annals of the N.Y. Acad. of Sci. 97:166

Glick, B.: 1955. Growth and function of the bursa of Fabricius. Poultry Sci. 34:1196.

———. 1960 White Pekin duck, White Leghorn, outbred and inbred New Hampshire. Poultry Sci. 39:130.

Godfrey, G. F.: 1952. Evidence for genetic variation in resistance to Newcastle disease in the domestic fowl. Jour. of Heredity 43 22.

Goodwin, K.: 1957. Subsequent viability and performance of Leghorns brooded adjacent to adult hens. Poultry Sci. 36:1122.

———, Dickerson, G. E., Lamoreux, W. F., Schaaf, K., and Urban, W. D.: 1956. The role of heterosis in resistance to respiratory lesions in the fowl. Poultry Sci. 35 915.

Gowe, R. S., Johnson, A. S., Crawford, R. D., Downs, J. H., Hill, A. T., Mountain, W. F., Pelletier, J. R., and Strain, J. H.: 1960. Restricted versus full-feeding during the growing period for egg production stock. Brit. Poultry Sci. 1:37.

———, Lemay, J. A., and Johnson, A. S.: 1962. The importance of genotype environment interactions for quantitative traits involving commercial egg production strains and two rearing environments—restricted and full-feeding. Proc. Twelfth World's Poultry Cong, Sydney, 34

———, and Wakely, W. J.: 1954. Environment and poultry breeding problems. I. The influence of several environments on the egg production and viability of different genotypes Poultry Sci 33 691.

Greenwood, A. W.: 1958 Long-term effects of a constant environment. Poultry Sci. 37:1208.

Guhl, A. M.: 1953. The social behavior of the domestic fowl. Kans. Agr. Exper. Sta. Tech. Bul 73:1

———. 1962. The social environment and behavior. In: The Behavior of Domestic Animals, ed. E S E Hafez, Baillière, Tindall and Cox, London.

———, Craig, J. V., and Mueller, C. D.: 1960. Selective breeding for aggressiveness in chickens. Poultry Sci 39:970.

Gyles, N. R., Kan, J., and Smith, R. M.: 1959. Heritability of breast blisters and breast feathering in a White Rock broiler strain. Poultry Sci. 38, 1210.

———, Kan, J., and Smith, R. M.: 1962. The heritability of breast blister condition and breast feather coverage in a White Rock broiler strain. Poultry Sci. 41:15.

Hendorf, A. J., Biewer, N. R., and Lamoreux, W. F.: 1947. The genetic relationship between mortality from induced and spontaneous lymphomatosis. Poultry Sci 26 67.

Hess, C. W., Edwards, H. M., Jr., and Dembnicki, E. F.: 1962. Growth rate selection on a methionine deficient diet. Poultry Sci. 41.1042

Hicks, A. F., Jr.: 1958. Genetic resistance to urine-nephritis in chickens. Poultry Sci. 37:1289.

———, and Lerner, I. M.: 1949. Hereditary crooked toes in chickens. Poultry Sci. 28 625.

Hill, C. H.: 1962. Nutritional factors influencing resistance and susceptibility to disease. Proceedings, Fifteenth Annual Calif. Animal Industry Conf., Univ. of Calif., Davis, Oct. 1962. 84.

Hollands, K. G., and Gowe, R. S.: 1961 The effect of restricted and full-feeding during confinement rearing on first and second year laying house performance Poultry Sci. 40 574.

Howes, C. E., and Hutt, F. B.: 1952. Breed resistance to nutritional encephalomalacia in the fowl. Poultry Sci. 31:390

———, and Hutt, F. B.: 1956. Genetic variation in efficiency of thiamine utilization by the domestic fowl. Poultry Sci. 35,1223.

Huston, T. M., Joiner, W. P., and Carmon, J. L.: 1957. Breed differences in egg production of the domestic fowl held at high environmental temperatures. Poultry Sci. 36,1247.

Hutt, F. B.: 1935. On the physiological basis of resistance to Salmonella pullorum in the fowl. Am. Naturalist 69:66

———. 1938. Genetics of the fowl. VII. Breed differences in susceptibility to extreme heat. Poultry Sci. 17:454.

———. 1949. Genetics of the Fowl. McGraw-Hill, New York.

———. 1958. Genetic Resistance to Disease in Domestic Animals. Constable & Co. Ltd., London, and Comstock Press, Ithaca, New York.

———. 1961a. Differential mortality in reciprocal crosses between Leghorns and heavy breeds. Poultry Sci. 40 1418.

————: 1961b. Identification and elimination of defects in animals. Germ Plasm Resources A.A.A.S., Washington, D.C., 355.

————, and Cole, R. K.: 1947. Genetic control of lymphomatosis in the fowl. Science 106:379.

————, and Cole, R. K.: 1948. The development of strains genetically resistant to avian lymphomatosis. Proc. Tenth World's Poultry Cong., Copenhagen, 719.

————, and Cole, R. K.: 1952. Heterosis in an inter-strain cross of White Leghorns. Poultry Sci. 31:365.

————, Cole, R. K., Ball, M., Bruckner, J. H., and Ball, R. F.: 1944. A relation between environment to two weeks of age and mortality from lymphomatosis in adult fowls. Poultry Sci. 23:396.

————, and Crawford, R. D.: 1960. On breeding chicks resistant to pullorum disease without exposure thereto. Canad. Jour. of Genetics & Cytology. 22:357.

————, and Scholes, J. C.: 1941. Genetics of the fowl. XIII. Breed susceptibility to Salmonella pullorum. Poultry Sci. 20:542

Hyre, H. N.: 1955. The effect of heredity and environment on keel deformities in White Leghorns. W. Va. Univ. Agr. Exper. Sta. Bul. 381.

Jaap, R. G.: 1958. Heritability, gene interaction and correlation for growth of glands associated with antibody formation of young chickens. Proc. Eleventh World's Poultry Cong., Mexico.

————: 1960. Heritabilities, gene interaction and correlations for growth of glands associated with antibody formation in the young chicken. Poultry Sci. 39:557.

Jaffe, W. P., and Payne, L. N.: 1962. The use of inbred lines of chickens in a study of the genetic basis of the graft-against-host reaction. Proc. Twelfth World's Poultry Cong., Sydney, J.

Jull, M. A.: 1952. Poultry Breeding. 3rd ed. John Wiley & Sons

Kheireldin, M. A., and Schaffner, C. S.: 1957. Familial differences in resistance to high environmental temperatures in chicks. Poultry Sci. 36 1334.

King, D. F., Cole, R. K., Hutt, F. B., and Cottier, G. J.: 1952. Tests in different environments of fowls genetically resistant to leucosis. Poultry Sci. 31:1027.

King, S. C., and Bruckner, J. H.: 1952. A comparative analysis of purebred and crossbred poultry. Poultry Sci. 31:1050.

Kondra, P. A., and Cavers, J. R.: 1947. Relation of the rate of feathering to the development of keel burnae. Poultry Sci. 26:85.

————, and Hodgson, G. C.: 1961. Genetic differences in energy-protein requirements of chickens. Poultry Sci. 40 525.

Lambert, W. V.: 1932. Natural resistance to disease in the chicken. I. The effect of selective breeding on natural resistance to fowl typhoid. Jour. Immunology 23:229.

————, and Knox, C. W.: 1932. Selection for resistance to fowl typhoid in the chicken with reference to its inheritance. Iowa Agr. Exper. Sta. Bul. 153 262.

Lamoreux, W. F., and Hutt, F. B.: 1939 Breed differences in resistance to a deficiency of vitamin B₁ in the fowl. Jour. Agr. Res. 58.307.

————, and Hutt, F. B.: 1948. Genetic resistance to deficiency of riboflavin in the chick. Poultry Sci. 27:334.

Landauer, W.: 1951. The hatchability of chicken eggs as influenced by environment and heredity. Storrs Agr. Exper. Sta. Bul. 262.

Lederberg, J., and Tatum, E L.: 1946 Gene recombination in Escherichia coli. Nature 158·558.

Lerner, I. M.: 1954. Genetic Homeostasis. Oliver & Boyd, Edinburgh.

————: 1958 The Genetic Basis of Selection. John Wiley & Sons, New York.

Lillie, R. J., and Bird, H. R.: 1949 A breed difference in feather pigmentation of vitamin D deficient chicks. Poultry Sci. 28:140

Lowry, D. C., Lerner, I. M., and Taylor, L. W.: 1956 Intraflock genetic merit under floor and cage managements. Poultry Sci. 35:1034.

Lush, J. L., Lamoreux, W. F., and Hazel, L. N.: 1948. The heritability of resistance to death in the fowl. Poultry Sci. 27:375

McBride, G.: 1958. The environment and annual breeding problems. Anim. Breed. Abs. 26:349.

————: 1960. Poultry husbandry and the peck order. Brit. Poultry Sci. 1:65.

————: 1962. The interactions between genotypes and housing environments in the domestic hen. Proc. Australian Soc. Anim. Prod. 4:95.

McClung, M. R.: 1961. The changing pattern of disease in the Rhode Island laying test. Poultry Sci. 40:1429.

McDonald, M. W.: 1957. Methionine supplements in chicken diets. II. A breed difference in growth response to DL methionine. Australian Jour. Agr. Res 8:587.

————: 1958. Methionine supplements in chicken diets III The biochemical difference in sulphur amino acid metabolism between White Leghorns and Australorps. Australian Jour. Agr. Res. 9:161.

————, and Bellharz, R. G.: 1962. Genetic variation in calcium metabolism in poultry. Proc. Twelfth World's Poultry Cong., Sydney, 88.

McNary, H. W., and Bell. A E.: 1957. An apparent maternal embryonic growth depressant in chickens. Poultry Sci. 36.1139.

———: 1961b. Identification and elimination of defects in animals. Germ Plasm Resources A.A.A.S., Washington, D.C., 555.

———, and Cole, R. K.: 1947. Genetic control of lymphomatosis in the fowl. Science 106:379.

———, and Cole, R. K.: 1948. The development of strains genetically resistant to avian lymphomatosis. Proc. Tenth World's Poultry Cong., Copenhagen, 719.

———, and Cole, R. K.: 1952. Heterosis in an inter-strain cross of White Leghorns. Poultry Sci. 31:365.

———, Cole, R. K., Ball, M., Bruckner, J. H., and Ball, R. F.: 1944. A relation between environment to two weeks of age and mortality from lymphomatosis in adult fowls. Poultry Sci. 23:396.

———, and Crawford, R. D.: 1960. On breeding chicks resistant to pullorum disease without exposure thereto. Canad. Jour. of Genetics & Cytology. 22:357.

———, and Scholes, J. C.: 1941. Genetics of the fowl. XIII. Breed susceptibility to Salmonella pullorum. Poultry Sci. 20:342.

Hyre, H. N.: 1955. The effect of heredity and environment on keel deformities in White Leghorns. W. Va. Univ. Agr. Exper. Sta. Bul. 381.

Jaap, R. G.: 1938. Heritability, gene interaction and correlation for growth of glands associated with antibody formation of young chickens. Proc. Eleventh World's Poultry Cong., Mexico.

———: 1960. Heritabilities, gene interaction and correlations for growth of glands associated with antibody formation in the young chicken. Poultry Sci 39 557.

Jaffe, W. P., and Payne, L. N.: 1962 The use of inbred lines of chickens in a study of the genetic basis of the graft-against-host reaction. Proc. Twelfth World's Poultry Cong., Sydney, 1.

Jull, M. A.: 1952. Poultry Breeding. 3rd ed. John Wiley & Sons.

Kheireldin, M. A., and Schaffner, C. S.: 1957 Familial differences in resistance to high environmental temperatures in chicks. Poultry Sci. 36:1334

King, D. F., Cole, R. K., Hutt, F. B., and Cottier, G. J.: 1952. Tests in different environments of fowls genetically resistant to leucosis. Poultry Sci. 31:1027.

King, S. C, and Bruckner, J. H.: 1952. A comparative analysis of purebred and crossbred poultry. Poultry Sci 31:1030.

Kondra, P. A., and Cavers, J. R.: 1947. Relation of the rate of feathering to the development of keel bursae. Poultry Sci. 26.83

———, and Hodgson, G. G: 1961. Genetic differences in energy-protein requirements of chicks. Poultry Sci. 40 522.

Lambert, W. V.: 1932. Natural resistance to disease in the chicken. 1 The effect of selective breeding on natural resistance to fowl typhoid. Jour. Immunology 23:229.

———, and Knox, G. W.: 1932. Selection for resistance to fowl typhoid in the chicken with reference to its inheritance. Iowa Agr. Exper. Sta Bul. 153:262.

Lamoreux, W. F., and Hutt, F. B.: 1939 Breed differences in resistance to a deficiency of vitamin B, in the fowl. Jour. Agr. Res. 58.307.

———, and Hutt, F. B.: 1948. Genetic resistance to deficiency of riboflavin in the chick. Poultry Sci. 27:334

Landauer, W.: 1951. The hatchability of chicken eggs as influenced by environment and heredity. Storrs Agr. Exper. Sta. Bul. 262.

Ledeberg, J., and Tatum, E. L.: 1946. Gene recombination in Escherichia coli. Nature 158:558

Lerner, I. M.: 1954. Genetic Homeostasis. Oliver & Boyd, Edinburgh.

———: 1958. The Genetic Basis of Selection. John Wiley & Sons, New York.

Lillie, R. J., and Bird, H. R.: 1949. A breed difference in feather pigmentation of vitamin D deficient chicks. Poultry Sci. 28.140.

Lowry, D. C., Lerner, I. M., and Taylor, L. W.: 1956. Intraflock genetic merit under floor and cage managements. Poultry Sci. 35:1034.

Lush, J. L., Lamoreux, W. F., and Hazel, L. N.: 1948. The heritability of resistance to death in the fowl. Poultry Sci. 27:375

McBride, G.: 1958. The environment and animal breeding problems. Anim Breed. Abs. 26:349

———: 1960. Poultry husbandry and the peck order. Brit. Poultry Sci. 1:65.

———: 1962. The interactions between genotypes and housing environments in the domestic hen. Proc. Australian Soc. Anim. Prod. 4:95.

McClung, M. R.: 1961. The changing pattern of disease in the Rhode Island laying test. Poultry Sci. 40:1429.

McDonald, M. W.: 1957. Methionine supplements in chicken diets. II. A breed difference in growth response to DL methionine. Australian Jour. Agr. Res. 8:587.

———: 1958. Methionine supplements in chicken diets. III. The biochemical difference in sulphur amino acid metabolism between White Leghorns and Australorps. Australian Jour. Agr. Res. 9:161.

———, and Bellharz, R. G.: 1962. Genetic variation in calcium metabolism in poultry. Proc. Twelfth World's Poultry Cong., Sydney, 83.

McNary, H. W, and Bell, A. E.: 1957. An apparent maternal embryonic growth depressant in chickens. Poultry Sci. 36:1159.

Markle, D. R.: 1939. Breeding poultry for viability. Tenn. Agr. Exper. Sta. Bul. 377.

Maw, A. J. G.: 1954. Inherited riboflavin deficiency in chicken eggs. Poultry Sci. 33:216.

Mullen, T. W., Hill, J. F., and Arvidson, R. B.: 1959. Inheritance of resistance to *Eimeria acervulina.* Poultry Sci. 38:1229.

Moultrie, F., Cottier, G. J., and King, D. F.: 1955. Additional evidence for genetic variation in resistance to "Blue Comb" disease. Poultry Sci. 34:458.

——, King, D. F., and Cotner, G. J.: 1953. The influence of heterosis and maternal effects on viability in an interstrain cross of White Leghorn. Poultry Sci. 32:935.

Nesheim, M. C., and Hutt, F. B.: 1962. Genetic differences among White Leghorn chicks in requirements of arginine. Science 137-691.

Newcomer, E. H.: 1959. Chromosomal translocation in domestic fowl induced by X-rays. Science 130.390.

Nordskog, A. W., and Johnson, E. L.: 1953. Breed differences in response to feeding antibiotics. Poultry Sci. 32 1046.

——, and Kempthorne, O.: 1958. Importance of genotype-environmental interactions in random sample poultry tests. Biometrical Genetics, Pergamon Press, N.Y.

——, and Phillips, R. E.: 1960. Heterosis in poultry. V. Reciprocal crosses involving Leghorns, heavy breeds, and Fayoumi. Poultry Sci. 39:257.

Oakberg, E. F.: 1951. Genetic differences in quantitative histology of the adrenal organ weight, and inter-organ correlations in White Leghorn chickens. Growth 15:57.

——, and Lucas, A. M.: 1949. Variation in body weight and organ: Body weight ratios of inbred lines of White Leghorn chickens in relation to mortality, especially from lymphomatosis. Growth 13 319.

Paterson, L. T., Johnson, L. W., and Edgar, S. A.: 1961. The comparative resistance of several inbred lines of S. C. White Leghorns to certain infectious diseases. Poultry Sci. 40:1442.

Polley, C. R., Grose, A. E., and Craig, J. V.: 1960. A skin grafting technique for use in genetic studies with chickens. Transplantation Bul. 7:425.

Ram, T., and Hutt, F. B.: 1955. The relative importance of body temperature and lymphocytes in genetic resistance to *Salmonella pullorum* in the fowl. Am. Jour. Vet. Res. 16:437.

Reid, W. M.: 1962. Comparative resistance of imported standard breeds and native Egyptian strains of poultry to *Ascaridia galli.* Poultry Sci. 34:30

Ritcher, P. O., and Inko, W. M.: 1948. External parasites of chickens and their control. Ky Agr. Exper. Sta. Bul. 517.

Roberts, E. F.: 1962. Genetic-nutritional interactions as affecting the early growth rate of chicken. Ph.D. Thesis on file at University of Minnesota Library.

Roberts, E. and Card, L. E.: 1935. Inheritance of resistance to bacterial infections in animals A genetic study of pullorum disease. III. Agr. Exper. Sta. Bul. 419:476.

Rosenberg, M. M., Alicata, J. E., and Palafox, A. L.: 1954. Further evidence of hereditary resistance and variability to cecal coccidiosis in chickens. Poultry Sci. 33 972.

Sadler, C. R., and Glick, B.: 1962. The relationship of the size of the bursa of Fabricius to antibody production. Poultry Sci. 41:508.

Sandler, L.: 1962a. Theory and instance of meiotic drive. Proc. Eleventh Nat. Poultry Breeders Roundtable. Kansas City, Mo. 72.

—— 1962b. Segregation distortion: A case of meiotic drive in natural populations of Drosophila. Proc. Eleventh National Poultry Breeders Roundtable. Kansas City, Mo. 72.

Schierman, L. W., and Nordskog A V: 1961. Relationship of blood type to histo-compatibility in chickens. Science 134·1008

——, and Nordskog A V: 1963. Influence of the B blood group histocompatibility locus in chickens on a graft versus host reaction. Nature 97·511.

Schultz, F. T., and Briles, W. E.: 1953. The adaptive value of blood group genes in chickens. Genetics 38 31.

Siegel, P. B., and Mueller, C. D.: 1955. The effect of low temperature exposure on inbred, outbred, and crossbred chicks.

——, and Wisman, E. L.: 1962. Protein and energy requirements of chicks selected for high and low body weight. Poultry Sci. 34:1415.

Shoffner, R. N.: 1948. Performance of crosses among lines of Leghorns. I. Hatchability. Poultry Sci 27,685

——, and Canfield, T. H.: 1957. Keel defects in males ranged with and without roosts. Poultry Sci 36 445

Sloan, H. J., Winters, L. M., Canfield, T. H., and Pilkey, A. M.: 1953. Development and performance of inbred lines of chickens. Minn. Agr. Exper. Sta. Tech. Bul. 207.

Smith, H. W.: 1956. The susceptibility of different breeds of chickens to experimental *Salmonella pullorum* infection. Poultry Sci. 35·701.

Sprent, J. F. A.: 1959. Parasitism, immunity and evolution. The evolutions of living organisms. Syn. Royal Soc. of Victoria, Melbourne (Vic.) Australia.

—— 1963 Parasitism: an introduction to parasitology and immunology for students of biology, veterinary science and medicine. Queensland University Press, Brisbane, Australia.

Stutts, E. C., Briles, W. E., and Kunkel, H. O.: 1956. Blood glutathione levels and egg production in inbred lines of chickens. Poultry Sci. 35:727.

———, Briles, W. E., and Kunkel, H. O.: 1957. Plasma alkaline phosphatase activity in mature inbred chickens. Poultry Sci. 36:269.

Taylor, L. W.: 1946. Multiplex combs. Poultry Sci. 25:610.

Lerner, I. M., Deome, R. B., and Beach, J. R.: 1943. Eight years of progeny-test selection for resistance and susceptibility to lymphomatosis. Poultry Sci. 22:339.

Thornton, P. A.: 1960. Thiamine requirements of growing chicks as influenced by breed differences. Poultry Sci. 39:440.

Tindell, D., and Craig, J. V.: 1959. Effects of social competition on laying house performance in the chicken. Poultry Sci. 38:95.

———, and Craig, J. V.: 1960. Genetic variation in social aggressiveness and competition effects between sire families in small flocks of chickens. Poultry Sci. 39:1318.

Warren, D. C.: 1937. Physiologic and genetic studies of crooked keels in chickens. Kans. State Agr. Exper. Sta Tech. Bul. 44.

———: 1942. The cross breeding of poultry. Kans. Agr. Exper Sta Bul. 52.

———: 1958 A half-century of advances in the genetics and breeding improvement of poultry. Poultry Sci. 37:3.

———, and Moore, C. H.: 1956. Adult mortality in reciprocal crosses of Leghorns and heavy breeds. Poultry Sci. 35:1178

Waters, N. F.: 1945. Breeding for resistance and susceptibility to avian lymphomatosis. Poultry Sci. 24:259.

———: 1949. The occurrence of crooked keels among inbred lines of White Leghorns. Poultry Sci. 28:725.

———: 1951. Mortality from lymphomatosis and other causes among inbred lines of White Leghorns. Poultry Sci. 30:531.

———: 1954a. Etiological relationship of visceral and neural lymphomatosis. Poultry Sci 33 365.

———: 1954b. Avian lymphomatosis mortality among inbred line crosses. Proc. Tenth World's Poultry Cong, Edinburgh, Scotland Aug 13. 2:201.

———, and Burmester, B. R.: 1961. Mode of inheritance of resistance to Rous sarcoma virus in chickens. Jour. National Cancer Inst. 27:655.

———, and Burmester, B. R.: 1963. Mode of inheritance of resistance to induced erythroblastosis in chickens Poultry Sci. 42:95.

———, Burmester, B. R., and Walter, W. G.: 1958. Genetics of experimentally induced erythroblastosis in chickens. Jour. National Cancer Inst. 20:1245.

———, and Bywaters, J. H.: 1949. Influence of age of chickens at contact exposure on incidence of lymphomatosis. Poultry Sci. 28:254.

———, and Bywaters, J H.: 1959 Poultry genetics as related to pathology. Chap. 3: Diseases of Poultry, 4th ed., Biester, H E. and Schwarte, L. H., Iowa State Univ. Press, Ames.

———, and Fontes, A. K.: 1960. Genetic response of inbred lines of chickens to Rous sarcoma virus. Jour. National Cancer Inst. 25:351.

———, Groscke, A. C., and Scott, H. M: 1950. Influence of diet on early mortality among inbred lines of chickens Poultry Sci 29.685.

Watson, J. D., and Crick, F. H. C.: 1953. Genetical implications of the structure of deoxynucleic acid. Nature 171:964.

Wilcox, F. H., Van Vleck, L. D, and Schaffner, C S: 1962. Serum alkaline phosphatase and egg production. Proc. Twelfth World's Poultry Cong. 19.

Wilson, J. E: 1958. Adeno-carcinomata in hens kept in a constant environment. Poultry Sci. 37:1253.

Zinder, N. D., and Lederberg, J.: 1952. Genetic exchange in Salmonella. Jour. of Bact. 64:679.

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4

Avian Hematology

Hematology is defined as that branch of biology which treats of the morphology of the blood and the blood-forming organs. When dealing with the variations of the blood, it is essential that one consider not only the cellular elements as they occur in the blood stream, but also the origin and relationship of the blood cells and the relations between blood cells and the cells of the connective tissues and the reticulo-endothelial system Many changes apparent in the peripheral blood are merely a manifestation of a reaction taking place in the blood-forming tissues themselves. Such changes should be studied at the site of primary disturbance in order to arrive at a clear understanding of the process. Poultry pathologists are becoming more concerned with diagnosis. These measurements are hindered by several factors in birds. Avian erythrocytes and thrombocytes are nucleated, and the blood clots very quickly. Hemoconcentration develops quickly in overheated, excited birds. Loss of blood as well as age, sex, season, egg production, and environmental conditions have been shown to alter the composition of blood.

The principal purpose of this chapter is to briefly outline the salient points of avian hematology. It will, therefore, be necessary to omit detailed discussion of many questions. Such discussions will be found in the references listed at the end of the chapter. The report of the conference on leukocyte functions contains a wealth of information of value to anyone interested in hematology (Gordon, 1955). The extensive atlas of Lucas and Jamroz (1961) beautifully illustrates the variety of blood cells in the chicken from embryonal age to adult as seen in film or imprint preparations. Their work is an excellent reference. The morphology and understanding of organization and relations of blood cells in tissues can be further developed from study of tissue sections under the light and electron microscopes. Correlation of all methods of study will lead to a sound knowledge

of the reactions of blood and blood-forming tissues in health and disease. The domestic fowl or chicken is the main subject considered in this chapter, and except where noted all discussion refers to it.

DESCRIPTIONS OF THE CELLS AND HEMOGLOBIN IN THE BLOOD

Numerous physiological factors influence the number of the various types of cells and the amount of hemoglobin found in the blood. For this reason it is not possible to give a single set of figures that may be regarded as fixed normal values. The data in Table 4.1 are to be regarded as approximate values for normal birds and should be used in connection with the knowledge available concerning physiological variations. The staining reactions of blood cells described here are those secured by the use of Wright's blood stain or the May-Grünwald and Giemsa combinations of blood stains.

Methods of Counting Blood Cells and Measuring Hemoglobin

Many procedures have been recommended for the enumeration of blood cells of birds. Since all blood cells of birds are nucleated, the methods commonly used for counting mammalian blood cells cannot be applied. Relatively little difficulty is encountered in counting erythrocytes, but the counting of leukocytes introduces certain problems. The main objection to the available methods for counting leukocytes is the relatively large error associated with them. This error can be partially compensated for by making duplicate or triplicate counts and using the arithmetic average as representative of the true count.

The direct method of counting the leukocytes suspended in a suitable medium (as Toisson's fluid) in the hemocytometer is useful and satisfactory when one is dealing with normal blood. It is difficult to distinguish pathological immature red

TABLE 4.1
COUNTS OF ERYTHROCYTES AND VALUE FOR HEMOGLOBIN IN THE BLOOD OF BIRDS

Bird	Sex	Erythrocytes *	Hemoglobin †	Method of Measuring Hemoglobin	Observer
Chicken (*Gallus domesticus*)..	Male	3 23	11 76	Photoelectric	Olson (1937)
	Female	2 72	9 11		
Duck (*Anas platyrhynchos platyrhynchos*) ...		3 06	15 6	Photoelectric	Magath and Higgins (1934)
Pigeon (*Columbia domestica*) ...	Male	3 228	15 97	Oxygen Capacity	Riddle and Braucher (1934)
	Female	3 096	14 72		
Dove (*Streptopelia risoria*)	Male	3.045	14 56		
	Female	2 989	13 97		
Turkey....			10.7	Newcomer	Dukes and Schwarte (1931)
Pheasant. ..			13 7		
Geese......			14 9		
Swan.			13 4		
Brants....			14 7		
Peafowl.			12.0		
Canary....	Female	4.516	9 5	Newcomer	Young (1937)

* Expressed in millions per mm.³
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Bird	Sex	Erythro-cytes[*]	Hemo-globin[†]	Method of Measuring Hemoglobin	Observer
Chicken (Gallus domesticus)...	Male	3.23	11.76	Photoelectric	Olson (1937)
	Female	2.72	9.11		
Duck (Anas platy-rhynchos platy-rhynchos)...		3.06	15.6	Photoelectric	Magath and Higgins (1934)
Pigeon (Columba domestica)...	Male	3.228	15.97	Oxygen Capacity	Riddle and Braucher (1934)
	Female	3.096	14.72		
Dove (Streptopelia risoria)	Male	3.045	14.56		
	Female	2.989	13.97		
Turkey ...			10.7	Newcomer	Dukes and Schwarte (1931)
Pheasant			13.7		
Geese....			14.9		
Swan...			13.4		
Brants.			14.7		
Peafowl			12.0		
Canary.	Female	4.516	9.5	Newcomer	Young (1937)

* Expressed in millions per mm.³
† Expressed in grams per 100 cc.

blood cells from leukocytes by this method. Under these conditions the direct method is obviously subject to error, and other methods are more suitable.

The method of Wiseman (1931) has been found to be fairly satisfactory for the routine study of chicken blood (Olson, 1935; Denington and Lucas, 1955). The modification of Mushett (1956) should be more accurate when the number of heterophils and eosinophils is low. Other methods have also been used (Diesem et al., 1958; Chubb and Rowell, 1959).

The number of thrombocytes may be estimated by counting the number of these cells which appear in the blood smear in conjunction with the 200 or more leukocytes observed in the process of making the differential leukocyte count. From the ratio thus found and the previously found total leukocyte count may be estimated the number of thrombocytes per cubic millimeter of blood.

The coefficients of variation or percentage error have been found in a series of counts of the blood cells to be approximately as follows (using phloxine diluting fluid):

Counts of erythrocytes   5.78 per cent
Counts of thrombocytes .   23.66 per cent
Counts of total leukocytes   34.2 per cent
Differential leukocyte counts:
Lymphocytes ....   8.6 per cent
Heterophils .. .. 27.96 per cent
Eosinophils .. .. .. .58.84 per cent
Basophils ....62.68 per cent
Monocytes ... ... .22.24 per cent

Each worker should determine the order of accuracy that he may expect with a single count of blood cells. The error will be reduced if two complete counts are made and the average of the two used to represent the count. Such a procedure is to be recommended.

Differential counts of the leukocytes and morphological studies of the blood cells may be made from blood smear preparations. Avian blood films should be spread quickly and evenly on clean slides previously heated in an alcohol flame to promote rapid drying. Wright's blood stain may be followed by Giemsa The basic staining elements (especially cell nuclei) of avian blood are much more numerous than those of mammalian blood; therefore, the technique usually employed for staining mammalian blood should be altered in order to obtain the best results with avian blood. No single staining procedure will be satisfactory in all circumstances. Different batches of staining solutions should always be tested by actual use and the staining time varied to suit the individual preparation. In the case of Wright's blood stain the length of time allowed for the stain to act may be varied. The staining time may also be varied with May-Grünwald stain. Giemsa stain may be varied both as to length of staining time and concentration The May-Grünwald and Giemsa combination has been found useful especially with pathological blood as in leukosis when there are many basic staining elements It is also invaluable for staining tissue imprint preparations. In such instances the concentration of the Giemsa solution should be increased.

The number of leukocytes may be expressed in terms of their relative number (percentage value as found in the differential count) or as an absolute number (actual number of cells per unit volume of blood, obtained by multiplying the total leukocyte count by the respective percentage value). The absolute count of the various types of leukocytes is a more reliable index to changes that may occur in an individual animal than the percentage value. A change may occur in the percentage value of one type of cell due to an increase or decrease of another type of leukocyte, although the absolute value of the first type of cell remains the same. For example, given a total count of leukocytes of 20,000 cells and the differential count values of 25 per cent heterophils and 60 per cent lymphocytes, the absolute count would be 5,000 heterophils and 12,000 lymphocytes. Another differ-

ential count might be 12.5 per cent hetero-
phils and 80 per cent lymphocytes, with
a total count of 40,000 leukocytes, which
at a glance might be taken to indicate
a decrease of heterophils by half and a
slight increase of lymphocytes. Actually,
the absolute values of the second count
would be 5,000 heterophils, or no change,
and 32,000 lymphocytes, an increase of
about two and a half times.

For practical purposes, hemoglobin
may be measured by one of the acid hema-
tin methods using a colorimeter or photel-
ometer (Bankowski, 1942). The mano-
metric method remains the most correct
procedure (Rostorfer, 1949).

Erythrocytes and Hemoglobin

The erythrocytes (red blood cells) of
the bird are oval and nucleated. Vari-
ations in shape may be noted in the blood
of a normal bird, and occasionally a spher-
ical erythrocyte may be noted. The
nuclei of the oval cells are likewise oval
and of a mature character. They have
relatively large irregular blocks of deep-
staining chromatin material that is dis-
tinct from the more lightly staining para-
chromatin. The cytoplasm is orange in
color. The round erythrocytes have a
round, slightly less mature nucleus with
smaller clumps of chromatin. Erythrocytes
without nuclei are occasionally seen. The
average dimensions of chicken erythro-
cytes, as found by Scarborough (1931–32)
in his review of the literature, were 12.2μ
by 7.3μ. Lucas and Jamroz (1961) found
their inbred laboratory chickens to have
smaller sized and a greater number of
erythrocytes than commercial stock and
also give data on erythrocytes of other
birds. Jaffe (1960) noted a lower number
of red blood cells, hemoglobin concentra-
tion, and hematocrit value in one of
inbred lines of chickens. The red blood
cells differ in size in different species in
birds, and in general they are larger in
the larger species. Melampy (1918) has
found nuclear material of the chicken
erythrocyte to comprise about one-fourth

the dry weight of the cell and has noted
considerable differences in distribution of
amino acids in cytoplasm and nucleus.
Two components of avian hemoglobin
were distinguished on the basis of amino
acid composition which were different
from any human hemoglobin (Van der
Helm and Huisman, 1958).

The count of erythrocytes and value
for hemoglobin are usually higher in male
birds than in female birds. This difference
does not become apparent until about the
time of sexual maturity. A hormonal in-
fluence is also indicated by the fact that
juvenile and gonadectomized chickens of
both sexes tend to have approximately the
same counts of red blood cells. Domm
and Taber (1946) have shown that
androgens (testosterone propionate) will
cause an increase of erythrocytes in capons
and five-month-old pullets. Younger pul-
lets did not respond as well, indicating
an age factor. Estrogen (alpha-estradiol
benzoate) in large amount tended to
counteract the effect of the androgen.
Large amounts of a synthetic estrogen
caused no change in erythrocytes or various
types of leukocytes of young adult hens
(Diesem et al., 1958). Thyroidectomy of
only male chickens caused a decrease of
erythrocytes, whereas thiouracil treatment
caused a decrease of erythrocytes in males,
females, and capons. The number of
erythrocytes and amount of hemoglobin
vary with the season, the lowest values
being found in the late summer and early
fall, and the highest values in the winter
(Olson, 1937). No seasonal effect was noted
in Hawaii by Tanaka and Rosenberg
(1954), who thought this might be due to
the even temperature of the climate.
Domm and Taber (1946), making obser-
vations at three-month intervals, reported
the lowest erythrocyte values for hens in
the winter and spring coinciding with egg
production. The highest values were ob-
served in the autumn. These workers
noted no seasonal variation of red blood
cells of males or capons. In pigeons and
doves the males have higher values than

the females; the lowest values for hemoglobin and erythrocytes are found in the summer; and the highest hemoglobin levels are noted in the winter, while the largest counts of erythrocytes are noted in the fall (Riddle and Braucher, 1934). A similar seasonal variation has been observed in canaries by Young (1937). Higher blood pressure in the winter than in the summer was noted in both male and female chickens by Weiss *et al.* (1961). This may be a factor related to the seasonal variation of erythrocytes. A 48-hour starvation period was found by Palmer and Biely (1935b) to increase the counts of red blood cells. A diurnal variation of erythrocytes with high values at midnight and low values at noon has been observed (Domm and Taber, 1946). Cook and Harmon (1933) stated that the amount of hemoglobin varied with the intensity of egg production. They did not consider the effect of season, and others (see Olson, 1937) have questioned this statement. Diet will influence the level of hemoglobin, as iron sulfate or casein tend to increase the value (Cook and Harmon, 1933, and Cook, 1937). Hogan and Parrott (1940) found an anemia-preventing factor in the vitamin B complex which later was termed B_c and found identical with folic acid (O'Dell and Hogan, 1943). Deficiency of this vitamin results in anemia, retardation of growth, and reduction of leukocytes and thrombocytes; a larger amount being necessary to maintain a normal level of leukocytes (Campbell *et al.*, 1944). Sturkie (1943) found no significant increase of hemoglobin following asphyxia in either splenectomized and normal hens and thus failed to support the previous report on reservoir function of the spleen by Harmon *et al.* (1932). Pigeons subjected to sudden lowering of environmental air pressure for 8 hours had increased counts of erythrocytes and values for hemoglobin (Kocian, 1936). The factor of indoor versus outdoor environment was found to have no effect on the number of erythrocytes or level of hemoglobin (Olson, 1937).

Measurement of blood cells packed by centrifugation (hematocrit) will provide an approximate index of the number of erythrocytes, and this has been applied in avian hematology. The hematocrit, in conjunction with the number of erythrocytes, is used to calculate the mean corpuscular volume. The hematocrit is not a proper substitute for a count of erythrocytes or a determination of hemoglobin. The color index is computed from these values. Relatively few observations have been made on the fragility of avian erythrocytes.

There are various antigens of the chicken red blood cell. Some can be detected by agglutination of the erythrocytes with bovine serum although specific antiserums prepared in the rabbit permit more detailed study. A comprehensive review of the subject is given by Briles (1960). Each antigen of the erythrocyte is controlled by a gene which belongs to one of seven presently recognized blood group systems. A great number of combinations can occur since all but two blood group loci appear to be located on separate chromosomes. Fanguy (1961) gives a good description of the details of typing.

Polymorphonuclear Heterophilic Granulocytes (Heterophils)

These cells are imperfectly round and have a diameter of approximately 10μ. Their characteristic feature is the presence of many acidophilic crystalline granules in a clear colorless cytoplasm. In the chicken these granules are usually rod- or spindle-shaped. Frequently, in routinely stained smears, especially when Wright's stain is used, these granules are distorted in shape and appear as round or short rod forms. Magath and Higgins (1934) found the granules to be round in the heterophils of the duck. The nucleus is lobulated, with fairly heavy bands connecting the lobes. The number of lobes is usually two or three, and occasionally single or imperfectly lobulated nuclei may be observed. The number of lobes in the

nucleus or Arneth count is sometimes used as a criterion of age of the heterophil. Thus a relatively large number of heterophils with single or few lobes indicates production of many young cells. A relatively large number of heterophils with many lobes indicates aging of the cells with low production of young cells. An impression of the relative number of lobes can be gained while examining a blood smear without actually making an Arneth count. The chromatin and parachromatin arrangement is relatively heavy and coarse in the nucleus. The heterophils function in the defense mechanism against bacterial invasion as phagocytes that can be rapidly mobilized. These cells also have bactericidal action and the power to digest protein.

Male chickens have a slightly higher percentage of heterophils than do females, amounting to approximately 5 per cent. Heterophils also tend to be more numerous in the blood of older birds (Olson, 1937). Considerable variation in the percentage of these cells may occur with the passage of time in a given individual; this variation is somewhat less if one considers the absolute count only. Shaw (1933) reported a diurnal rhythm of the leukocytes of the pigeon. The relative count of lymphocytes is higher than the relative count of heterophils in the morning, but in the afternoon the relative count of heterophils may be greater, equal to, or slightly less than that of the lymphocytes. This is due to an increase in the absolute number of heterophils during the afternoon while the absolute count of lymphocytes remains constant.

Polymorphonucleor Eosinophilic Gronulocytes (Eosinophils)

The eosinophils are of about the same size as the heterophils. They possess relatively large spherical granules whose color is dull red in contrast to those of the heterophil. Magath and Higgins (1934) found the granules of the duck's eosinophil to be rod-shaped. The cytoplasm has a faint bluish-gray tint. The nucleus is often

bilobed, and the chromatin appears to be stained a richer blue than in the heterophil nucleus. The functions of eosinophils are not well understood. It is suspected that they act as a detoxifying power. In some animals and birds they are increased in verminous infestations and are found in the tissues in certain allergic states.

Polymorphonucleor Basophilic Gronulocytes (Basophils)

The basophils are of about the same size and shape as the heterophils. The nucleus is usually masked by the mass of granules in the cells. It is weakly stained, and round or oval in shape, although often it is lobulated. The cytoplasm is clear and colorless. Dark-staining, moderate-sized, basophilic granules are abundant. The material composing the granules is water soluble and may be washed from the cell. More frequently the basophilic material is incompletely washed, and the granules appear distorted and broken up. The function or functions of basophils or their tissue counterpart, the mast cells, are not known despite considerable investigation and many theories. Although there are relatively few basophils in the blood stream of the chicken, tissue mast cells are numerous. It is believed that the tissue mast cell may enter the blood stream and be identical with the basophil, and the reverse, that is, basophils leaving the circulation to become mast cells in the tissues, is considered likely in the chicken.

The number of basophils normally present in the blood is small. A slightly greater number may be found in the blood of young chicks than in the blood of adults.

Lymphocytes

The lymphocytes constitute the majority of leukocytes in the blood of the fowl. There is a wide range in the size and shape of these cells. In the past there has been a tendency to classify lymphocytes on the basis . . . into large, medium, and small

of that individual. These workers (1935b) have also reported that there is an increase of leukocytes amounting to about 25 per cent, after a 48-hour fast. Hoppe (1935) noted a digestive leukocytosis with a maximum number of cells 4 to 5 hours after feeding, but only when the feeding period had been preceded by a 24-hour fast. Cook (1937) states that high counts of leukocytes are commonly found in chickens fed diets in which there is a lack of antihemorrhagic factor or minimal amounts of nitrogenous bases. Brief exposure to high temperatures (15–30 minutes at 112–118° F.) caused a decrease in leukocyte numbers (Chancellor and Glick, 1960).

Thrombocytes

These are the smallest cells seen in the blood of the fowl. They vary considerably in size and form. The typical thrombocyte is oval with a more nearly round nucleus in the center of a clear cytoplasm. There are two or three small, brightly red-staining granules at one pole of the cell. The chromatin of the nucleus is dense and is clumped into relatively coarse masses which are distinctly separated by the parachromatin. The thrombocytes are generally believed to play a part in the coagulation of the blood.

Thrombocytes are slightly more numerous in female than in male adult chickens and also more numerous in young than in adult chickens.

The number of various types of cells and the amounts of hemoglobin in the blood of normal birds are listed in Tables 4.1 and 4.2, representing the values reported in the literature. It is impossible to give a set of meaningful values for blood cells that will fit all circumstances and conditions. Some of the problems involved are discussed in detail by Lucas and Jamroz (1961). The biologist who seeks a normal base line with which to compare his observations should not be discouraged but realize that this is only nature's challenge of his ability to establish adequate and proper controls for his specific experiments.

THE ORIGIN OF BLOOD CELLS

The following discussion of origin of blood cells and relationships in tissues is a generalization. The sources of the information are listed in the references and

TABLE 4.2

COUNTS OF THROMBOCYTES AND LEUKOCYTES IN THE BLOOD OF BIRDS

Bird	Sex	Thrombocytes*	Leukocytes*	Differential Counts (Percentage)					Observer
				Lymphocytes	Heterophils	Eosinophils	Basophils	Monocytes	
Chicken (Gallus domesticus)..	M	25.4	19.8	59.1	27.2	1.9	1.7	10.2	Olson (1937)
	F	26.5	19.8	64.6	22.8	1.9	1.7	8.9	
Duck (Anas platyrhynchos platyrhynchos)..	..	30.7	23.4	61.7	24.3	2.1	1.5	10.8	Magath and Higgins (1934) Shaw (1933)
Pigeon A.M...			13.05	65.6	23.0	2.2	2.6	6.5	Johnson and Lange (1939)
P.M...			18.55	47.8	42.8	1.9	2.4	5.1	
Turkey				50.6	43.4	0.9	3.2	1.9	

* Expressed in thousands per mm³

lymphocytes. Such a classification is entirely arbitrary as there are no sharply defined distinctions between such groups. The cytoplasm is usually weakly basophilic and may be confined to a narrow rim bordering one side of the nucleus, or it may constitute the major portion of the cell as in the case of the larger lymphocytes. The nucleus is usually round and may be slightly indented at one side. The chromatin pattern is usually rather coarse and blocky, especially in the small, more mature type of cells. In some instances the chromatin is rather fine and is not distinctly separated by the parachromatin material. Occasionally a few nonspecific azure granules may be noted in the cytoplasm especially near the point of indentation of the nucleus. It has been suggested with the support of some evidence that lymphocytes are capable of fixing toxic material and thus acting as a protective mechanism. Due to their high lipase content it has also been suggested that they participate in the digestion of fat. Studies in mammals have indicated that there are more lymphocytes entering the blood stream in 24 hours than are found in the circulation at any one time, which suggests a rather active circulation of these cells from the blood to the tissues and back to the blood again. Lymphocytes are abundant in the wall of the intestine, and many probably pass into the lumen and are lost. Lymphocytes in the tissues may differentiate into various types of cells. Mononuclear leukocytes or polyblasts are found forming a protective barrier between foci of chronic inflammation and healthy tissue. These cells are derived from the blood lymphocyte. It has been demonstrated that the resistance of mice to growth of inoculated cancer cells is directly related to the activity of lymphoid tissue.

The number of lymphocytes in the blood of adult female chickens is slightly greater than in the blood of the adult male chickens. They are also somewhat more numerous in the blood of young birds than that of adult chickens.

Monocytes

The monocytes of the fowl are sometimes difficult to distinguish from the larger lymphocytes, and transitional forms between the two types of cells appear to exist in the blood. Monocytes are large cells with relatively more cytoplasm than the large lymphocytes. The cytoplasm has a blue-gray tint. The nucleus is rather irregular in outline. The nuclear pattern is usually of a more delicate composition than in the lymphocyte, the chromatin having the tendency to appear in the form of strands rather than blocks. The functions of monocytes in the blood are not well understood. It is rather generally accepted that these cells may migrate into inflamed tissues and there hypertrophy to form large active phagocytes. They may then engulf not only bacteria but also particulate matter such as cellular debris; they also have the ability to digest such material. The monocyte is probably capable of differentiation into fibroblasts in the tissues.

Monocytes are more numerous in the blood of adult male chickens than that of adult female chickens. They are also more numerous in the blood of chickens kept in an outdoor environment than that of those confined indoors.

Total Leukocyte Counts

The various factors influencing the number of specific types of leukocytes will obviously affect the total count of these cells. Some such physiological factors have been discussed above and will not be considered here. The total number of leukocytes in the blood is greater in young chickens than in adult birds. Adult chickens kept in an outdoor environment have higher total leukocyte counts than those kept indoors (Olson, 1937). Palmer and Biely (1935d) reported that the counts of leukocytes tended to be high in birds kept in strict confinement. According to Palmer and Biely (1935a), the number of leukocytes in the blood of a chicken tends to fluctuate around a particular level characteristic

1   2   3   4   5   6   7   8   9   10   11   12

13

14   15   16   17   18

19   20   21   22   23   24   25   26   27

28   29   30   31   32   33

34   35   36   40   41   43   44

37   38   39   42   45   46

47   50   52   55   57   61
48   51   53   56   58
49   54   59   60   62

10 μ

C. Olson

FIGURE 4.1

The cells illustrated were selected from dry smear preparations of blood or bone marrow and stained with May-Grünwald and Giemsa combinations unless otherwise indicated. Cells 1 through 12 represent the developmental stages of the erythrocyte. Cells 13 through 27 represent stages of development of the granulocyte. Cells 23 through 46 (except 42) are different types of mature leukocytes. The source of the cells has no special implication except to emphasize the fact that they occur and can be recognized under diverse conditions. For example, cell 3, a lymphoid erythroblast, is not ordinarily found in the circulating blood of lymphocytoma and was probably pushed into the circulation by growth of a focus of the tumor in the marrow.

1. Proerythroblast from marrow, transmissible granuloblastic leukosis. Wright's stain.
2. Lymphoid erythroblast from blood, transmissible erythroblastic leukosis. Wright's stain.
3. Lymphoid erythroblast from blood with leukemoid reaction, spontaneous lymphocytoma.
4. Polychrome erythroblast from blood, spontaneous erythroblastic leukosis.
5, 6, and 7. Polychrome erythrocytes from blood, spontaneous erythroblastic leukosis.
8, 9, 10, and 12. Erythrocytes from blood, normal.
11. Erythrocyte of blood from which nucleus has become lost.
13. Lymphoid stem cell or myeloblast from blood with leukemoid reaction, spontaneous lymphocytoma.
14 and 15. Lymphoid stem cells or myeloblasts from marrow, transmissible granuloblastic leukosis. Wright's stain.
16 and 18. Leukoblasts from blood with leukemoid reaction, spontaneous lymphocytoma.
17. Leukoblasts of Rieder type with lobulated nucleus from blood, transmissible granuloblastic leukosis, Wright's stain.
19 and 20. Metamyelocytes from blood with leukemoid reaction, spontaneous lymphocytoma.
21. Myelocyte from blood with leukemoid reaction, spontaneous lymphocytoma.
22. Myelocyte from blood, spontaneous erythroblastic leukosis.
23. Heterophil granulocyte with variation of granules from blood.
24, 25, 26, and 27. Heterophil granulocytes, normal. Cell 27 shows swelling and rounding of granules as an artifact due to staining reaction.
28, 29, and 30. Eosinophilic granulocytes, normal.
31, 32, and 33. Basophilic granulocytes, normal. Cell 33 shows loss of water-soluble basophilic material from granules.
34, 35, 36, 37, 38, and 39. Lymphocytes, normal.
40 and 41. Thrombocytes, normal.
42. Mitotic figure in immature cell from blood with leukemoid reaction, spontaneous lymphocytoma.
43, 44, 45, and 46. Monocytes, normal.
47 to 54. Plasmodium gallinaceum parasites in erythrocytes of chickens. Blood films through courtesy of the late Dr. F. R. Beaudette.
55 to 59. Haemoproteus parasites in erythrocytes of turkeys. Blood films through courtesy of the late Dr. F. R. Beaudette.
60, 61, and 62. Leucocytozoon smithi and erythrocyte, blood of turkey. Blood films through courtesy of the late Dr. E. P. Johnson, Blacksburg, Va.

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5, 6, and 7. Polychrome erythrocytes from blood, spontaneous erythroblastic leukosis.

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17. Leukoblasts of Rieder type with lobulated nucleus from blood, transmissible granuloblastic leukemia. Wright's stain.

19 and 20. Metamyelocytes from blood with leukemoid reaction, spontaneous lymphocytoma.

21. Myelocyte from blood with leukemoid reaction, spontaneous lymphocytoma.

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on the twelfth day, confirming the observation of Ackerman and Knouff (1959), the bursa on the fifteenth day with lymphoid cells almost entirely confined to these organs by the eighteenth day of incubation. The spleen was involved almost exclusively in granulopoiesis and liver and bone marrow in erythropoiesis at that time. Soon after hatching, significant lymphoid tissue was noted in the spleen and intestine.

The formation of blood cells in the adult proceeds in a somewhat different manner from that outlined for the embryo. However, in times of stress upon the hematopoietic system or in some primary pathological conditions, there may be a partial return to the embryonic type of hematopoiesis. Erythrocytes and granulocytes are formed principally in the bone marrow. The erythrocytes are developed within sinuses lined by endothelial cells. Under normal conditions the young and immature erythrocytes are held within the sinuses during their development. When mature they are released to the circulation. Evidence indicates that the sinuses are in open communication with the blood circulation; however, the mechanism by which the immature cells are retained within the sinuses until maturity is not known. Granulocytes are formed principally in the bone marrow. Their locus of development is in the intersinusoidal areas and, therefore, extravascular. Under normal conditions they usually develop by multiplication of leukoblasts and premyelocytes, cell types already partially differentiated and fixed in their line of development. When the granulocytes have matured sufficiently they enter the circulation by migration into adjacent sinusoids. The myeloblast or hemocytoblast represents an ancestral cell type common to both granulocytes and erythrocytes. These cells occur in the marrow and according to Jordan (1936) are represented by the germinal cells in the lymphoid nodules. His work indicates that the germinal cells produce small lymphocytes which surround them as a mantle. The lymphoid hemoblasts in the marrow may become either

granulocytes or erythrocytes, depending on whether or not they gain access to the sinusoids before undergoing further development. The lymphoid hemoblasts or small lymphocytes may also form thromboblasts which multiply in the sinusoids and later become thrombocytes. Jordan and Robeson (1942) believe the spleen is a normal source of the small lymphocytes in young pigeons. They demonstrated development of lymphoid foci in the marrow similar to those of the spleen as a result of splenectomy. This increase of lymphoid tissue supplied the deficiency resulting from loss of the spleen.

The source of blood lymphocytes is chiefly the diffuse lymphoid tissue scattered along the intestinal tract, in the liver, and in the spleen, together with the more or less organized nodular lymphoid tissue of the spleen, thymus, cecums, and palatine tonsils. Jordan (1936) believes that the small lymphocyte or the bone marrow (lymphoid hemoblast) is identical with that of the circulating blood. The circulating lymphocyte of the blood may then be regarded as a potential hemoblast. Likewise, the germinal center cells in the organized nodular lymphoid tissue are hemocytoblasts.

THE RELATIONSHIPS OF BLOOD CELLS IN THE TISSUES

A consideration of the blood cells in the tissues leads to one of the most fascinating aspects of hematology, as well as to one of the most confusing. With the exception of the erythrocytes, all cells of the blood have important functions in the tissues outside of the blood stream under normal as well as pathological conditions. Such cells as the heterophils fulfil their function in the tissues without change. The mononuclear leukocytes (lymphocytes and monocytes) are endowed with the ability of transformation into other types of cells in the tissues. Studies of such transformations have been made by various means. Morphology alone is a useful and important method of study, but it does not indicate function as well as does physiological study by

means of tissue culture methods, supravital and intravital staining, and observations on the property of phagocytosis. The tissue cells of mammals have been studied more extensively than those of birds; however, it has been shown that analogous cell types exist in the chicken. This discussion is, therefore, rather general in nature.

Aschoff grouped an extensive and widespread system of phagocytic cells under the term reticulo-endothelium. The elements of this system are most numerous in the blood-forming organs. The cells belonging to this system have been given various names (fixed and free macrophages, clasmatocytes, Kupffer cells, active and resting wandering cells, histoblasts, littoral cells, histiocytes, adventitial cells, alveolar phagocytes of the lung, and others). Mann and Higgins (1938) suggest the use of the term histiocyte to designate the cells of this system. Thus there are the fixed histiocytes representing that portion of the system which lines the vascular sinusoids, such as the littoral cells of the liver, spleen, organized lymphoid tissue, and bone marrow. The free histiocytes include those elements of the reticulo-endothelial system found free in the tissues. The cells of the reticulo-endothelial system, the blood cells, and the fibroblastic cells have a common origin from the mesenchyme. Under many conditions they react as a group to protect the body from injury. The reticulo-endothelial system is in addition associated with the metabolism of iron, lipids, carbohydrates, and proteins, and the destruction of red blood cells as well as other processes of the body.

The free histiocytes of the tissues have many morphological variations. The term polyblast was suggested by Maximow as descriptive of the histiocytes observed in areas of inflammation. The free histiocytes are regarded as being derived principally from the lymphocytes and monocytes of the blood. Some may be formed by cell division of histiocytes and others by differentiation of fibroblastic elements. Some fixed histiocytes may become free and active. Transitional forms of histiocytes resembling fibroblasts are found in the tissues, suggesting the development of such cells into fibroblasts or the reverse. Both processes have been observed in tissue cultures. It should be pointed out that tissue cultures of fibroblasts indicate the existence of different races and that the physiological properties and developmental tendency of such cells depend upon their origin and environment.

The fixed histiocytes are numerous in the sinusoids of the liver and spleen of the chicken where they actively phagocytize foreign particulate material. They exist in the lung as alveolar phagocytes, in the central nervous system as microglia, and in the loose connective tissue as sessile cells attached to the connective tissue fibrils. The fixed histiocytes may occur in any organ in the form of adventitial cells, in the adventitia of small blood vessels, and about the capillaries.

There is considerable variation in the amount of lymphoid tissue in the organs of normal chickens. The thymus and bursa of Fabricius increase rapidly in size after hatching of the chick and retrogress by the time of sexual maturity. Glick (1956) found that the bursa reached maximum size at four and one-half to six weeks in White Leghorn and ten to twelve weeks in Barred cross chickens. Cortisone (Glick, 1959), ACTH, and stress (Morita and Nishida, 1956; Newcomer and Connally, 1960) caused a decrease in size of the bursa of Fabricius. The lymphoid tissue in the spleen, wall of the intestine, and periportal areas of the liver varies in amount. Thorbecke et al. (1957) reviewed the literature on distribution of lymphoid tissue and found less development of lymphoid tissue in germ-free chickens. Mural lymphoid nodules (0.1 to 2.5 mm.) located at irregular intervals along lymph vessels and particularly abundant in the legs are described by Biggs (1957). A procedure for staining the dissected lymph vessels was required to locate the nodules. Myeloid metaplasia is a term usually used to indicate the formation of granulocytes in

organs other than the bone marrow. Such myeloid metaplasia may sometimes be observed in the lymphoid tissue of the thymus, periportal areas of the liver, and wall of the intestine. Huff and Bloom (1935) found marked extramedullary granulopoiesis and erythropoiesis in the spleen, liver, and kidney of canaries affected with malaria. The factors which control the amount of lymphoid tissue and degree of myeloid metaplasia are not well understood. In general it appears that age is one controlling element, as the lymphoid tissue is less active in older birds.

The role of the thymus, bursa of Fabricius, and spleen in development of immunity has attracted much attention in the past few years. Simonsen (1957) demonstrated that cells from the blood or spleen of a chicken mature enough to produce antibodies can be transplanted to the spleen of an embryo where they will grow and produce antibodies against their host. This results in a severe hemolytic anemia usually fatal in the second or third week after hatching. This is the reverse situation from actively acquired immunological tolerance in which the host cannot produce antibodies against foreign homologous cells when the host was exposed to these foreign cells in embryonal or neonatal life (Billingham et al., 1954). Bursectomized chickens have a reduced ability to produce antibodies (Glick et al., 1956; Mueller et al., 1962). Chicks hatched from eggs treated with 19 nortestosterone on the fifth or twelfth day of incubation have no bursa of Fabricius, a reduced thymus and spleen, and form antibodies poorly (Mueller et al., 1962). Hilgard et al. (1962), measuring the tolerance by graft of lymphocytes on chorioallantoic membranes, found that the tolerance decreases rapidly with age in most chickens but may last indefinitely in some. The chick with no bursa had little resistance to injection of adult homologous spleen cells which grew markedly in the spleen, peritoneal cavity, and subcutis (Papermaster et al., 1962). Because of the sequence of lymphoid development, Paper-

master and Good (1962) suggest that immunologically competent lymphoid cells may arise in the bursa and thymus, then populate the spleen, bone marrow, and other sites. Szenberg and Warner (1962) used three tests of immunological competence of chicks treated with testosterone in embryonal life. Some of the chicks had no bursa but would still reject a skin graft. Others had no bursa and complete atrophy of thymus cortex and would accept homologous skin graft. Both groups did not produce a circulating antibody or show delayed hypersensitivity to tuberculin. They concluded that the bursa is primarily responsible for production of a circulating antibody and delayed hypersensitivity, and the thymus is involved in host reaction to skin graft.

THE BLOOD IN DISEASE

Anemia is a condition in which the blood is deficient in either the number of erythrocytes or the amount of hemoglobin or both. The term is also sometimes applied when the erythrocytes show abnormal morphological features. Anemia may be associated with either acute or chronic disease, due either to suppression of blood cell production or to toxic destruction of blood cells. It may follow the loss of blood. Wirth and Kubasta (1939) have demonstrated the regenerative ability of the blood-forming tissues of the normal chicken by removing up to 85 per cent of the blood and observing the return of erythrocytes to normal levels within eight to nine days. The blood-forming organs of birds affected with disease will not respond with such rapidity. Anemia may result from the lack of constituents necessary for blood cell production, as in dietary deficiencies. The erythrocytes in anemia may show morphological deviations from the normal. The individual cells may be either larger or smaller than normal (anisocytosis). They may assume a variety of shapes such as being pointed at one end, or round (poikilocytosis). Immature erythrocytes may be found in large numbers in the blood, depending upon the

rate of production in the marrow. Many such immature cells are deficient in hemoglobin as indicated by the color of the cytoplasm which is blue-gray-yellow (polychromasia). It is important to distinguish between the blood picture of a severe regenerative anemia and that observed in erythroblastic leukosis. In the latter instance one may usually find the progenitors of the erythrocyte (lymphoid cells and lymphoid erythroblasts) in the blood. These are not usually present in the blood in simple anemia. Ishiguro (1957) has described a transmissible anemia which might be an anemic form of erythroleukosis. Study of bone marrow would aid in such a situation.

Leukocytosis is an increase in the number of leukocytes in the blood. Leukopenia is a decrease in the number of leukocytes in the blood. As has been mentioned, there is considerable variation in the number of leukocytes in the blood of normal chickens. In many disease conditions, especially bacterial infections, there may be a leukocytosis. Various irritants may act in different ways. Some may attract a specific type of cell and repel others. Some toxic materials may stimulate the blood-forming tissues to production of nearly all types of leukocytes. Others may suppress the production of leukocytes and lead to a condition of leukopenia. Myelocytes containing preacidophilic granules that take a basic stain are described as occurring normally in the blood of the ostrich (Jackson, 1936). In marked heterophilic leukocytosis there may be myelocytes in the peripheral blood. Such a condition should not be mistaken for leukemia as it merely represents the tremendous effort on the part of the bone marrow to fulfil the demand for heterophils that do not have time to ripen before being pushed into the blood stream.

Anemia and leukocytosis may accompany many bacterial diseases. The study of the blood picture cannot be relied upon to serve as a differential diagnostic test in the group of infectious bacterial diseases. The increase should be regarded as an index of the response of the defense mechanism of the host against the infection. A study of the blood and blood-forming tissues is essential for the diagnosis and differentiation of leukosis from other diseases of the fowl. Infections with Salmonella species of bacteria provoke a marked heterophilic leukocytosis in chickens (Moore, 1895–96; Taylor, 1916; Cook and Dearstyne, 1934; Olson and Goethius, 1937; and Wai and Stafseth, 1950). Anemia also is commonly present in such bacterial infections. Gauger et al. (1940) found no difference between the counts of erythrocytes and leukocytes in the blood of pigeons that were positive to a serological test for paratyphoid infection and those that were negative. Buxton (1960) has observed a severe hemolytic anemia in the last three days of acute, fatal fowl typhoid which appears to be due to sensitization of erythrocytes with bacterial polysaccharide and increased activity of the reticulo-endothelial system. In chronic fowl typhoid, the hemoglobin is decreased but not to such low levels. Ward (1904) has reported anemia and increased numbers of leukocytes in chickens with fowl cholera. Chickens spontaneously affected with tubercolosis have been found to have anemia and a leukocytosis of heterophils and monocytes (Olson and Feldman, 1936). Grimal (1938) studied the blood in two experimentally produced cases of the acute (Yersin) type of tuberculosis. A relative leukocytosis of lymphocytes was noted in the first week and of monocytes in the second, with a terminal relative leukocytosis of heterophils. These birds died 23 and 25 days after inoculation. Pomeroy and Fenstermacher (1937) described the findings in the blood of two turkeys with hemorrhagic enteritis. They observed anemia in both and a heterophilic leukocytosis in one. Wannop (1961) observed a decrease of erythrocytes and an increase of heterophils and monocytes in turkeys with "X" disease but commented that the same changes had been noted in other diseases of turkeys. Seastone (1935) observed a marked

monocytosis in chickens with experimentally produced listeriosis. Following intravenous inoculation of the bacteria, the monocytes increased from about 5,000 per mm.^{3} to a peak of 60,000 per mm.^{3} in five days, and at eight days dropped to about 10,000 per mm.^{3} There was only a moderate increase of granulocytes and apparently no disturbance of the lymphocytes.

Hemoglobin, erythrocyte, and leukocyte (total and relative values) measurements were essentially similar in experimental infectious synovitis whether caused by an agent or by pleuropneumonia-like organisms (Olson et al., 1957). There was anemia. The leukocytes increased from twenty to eighty thousand in 12 days and remained so for about 33 days. The absolute values were not given but there appeared to be an increase of heterophils, immature cells, and monocytes with perhaps a moderate increase of lymphocytes.

Thorp and Graham (1932) reported the results of counts of erythrocytes and leukocytes made on the blood of 71 chickens affected with acute laryngotracheitis. They found the number of erythrocytes to be within normal limits and the number of leukocytes to be slightly lower than the normal values for these cells as reported by other workers. It is pertinent to note that Thorp and Graham (1932) used the Blain method for counting leukocytes. This method has been observed to have a tendency to produce lower counts of leukocytes than other methods. In some cases of Newcastle disease, DeKoch (1954) observed a monocytosis and reduction of small lymphocytes. Many of the circulating monocytes contained phagocytosed red cells. The bone marrow was not affected, but degeneration and depletion of lymphocytes and an increase of reticulum cells and plasma cells were marked in the lymphoid deposits of the spleen, liver, and intestinal tract. Fredrickson and Chute (1958) noted a decrease of all leukocytes on the eleventh day of Newcastle infection and an increase of monocytes and heterophils on the fourteenth day. The sedimentation rate, plasma specific gravity,

and values for buffy coat were also altered during the disease. Sharma and Seetharaman (1950) observed anemia, a slight heterophil leukocytosis, and lymphopenia (to a calculated one-third pre-exposure level) in adult chickens 3 and 4 days after infection with Ranikhet virus.

An increase of eosinophils has been noted in the blood of a chicken with coccidiosis and tapeworms (Yakimoff and Rastégaieff, 1929), in a grouse harboring Trichostrongylus pergracilis (Fantham, 1912), and in chickens with Capillaria columbae infection (Olson and Levine, 1939). A leukocytosis of heterophils and slight anemia were also noted in Capillaria columbae infection. Chickens with coccidiosis have been observed to have a relative heterophilic leukocytosis (Fantham, 1912) and an increase in the total number of leukocytes (Krömker, 1937). A controlled infection of chickens with Heterakis gallinae demonstrated an increase of heterophils and eosinophils, though the increase was not related to the number of parasites present (Wickware, 1947). Experimentally produced and natural cases of typhlohepatitis (Histomonas meleagridis infection) in turkeys were found by Johnson and Lange (1939) to be associated with a relative heterophilia and monocytosis. Except for a slight anemia of uncertain significance, no changes of the blood were observed by Olson (1935) in chickens before and after treatment for heavy infestations of lice. Yakimoff and Rastégaieff (1930) have noted a decrease in the percentage of lymphocytes and an increase in heterophils together with a slight relative decrease in monocytes in chickens experimentally afflicted with spirochetosis. They also have observed a heterophilic leukocytosis in two spontaneous cases of spirochetosis. Coles (1939) states that a marked anemia may occur in young chicks with aegyptianellosis, but that the infection in adult birds is associated with only a transient anemia. Jacobi (1939) found that the number of erythrocytes dropped to about one-third the normal level during the acute stage of experimental infection

with *Plasmodium gallinaceum;* later the erythrocytes and hemoglobin were slowly and simultaneously regenerated to their normal levels. Rostorfer and Rigdon (1946) have shown the anemia of ducks with experimental acute malaria to be of the macrocytic hypochromic type. There was also a decrease in functional hemoglobin in relation to the hemoglobin measured as acid hematin.

Krömker (1937) reported anemia to be associated with gout. He also studied the blood of a chicken with fowl pox and found an increase in the total number of leukocytes at the time the skin lesions were beginning to dry.

The work of Goff et al. (1953) suggests that a heterophil leukocytosis, increased hematocrit and mean corpuscular volume with decreased corpuscular hemoglobin may be used to diagnose an early or borderline deficiency of riboflavin in young chickens. These changes returned to normal when riboflavin was supplied or after birds reached 12 weeks of age. Urethane, X-ray, and aminopterin decreased the number of circulating leukocytes (Dinning et al., 1950).

Vitamin K is related to clotting of the blood, and Griminger and co-workers (1953) found that it would reduce the clotting time prolonged by terramycin, arsenic, and arsanilic acid. Gray et al., (1954) studied the blood in 20 cases of hemorrhagic disease, in which growing chickens died with hemorrhages principally in skeletal and cardiac muscles, subcutis, and intestine. The clotting time was prolonged and the thrombocytes were enlarged and vacuolated. The number of heterophils varied but anemia was quite constant. The marrow was aplastic and a leukopenia existed near the time of death. Essentially similar findings in a larger number of cases were reported by Cover et al. (1955), who differentiated the condition from vitamin K deficiency. The aplastic anemia problem continues to exist with no new knowledge of its etiology (Hanley, 1962).

Increased coagulation time of blood and

decrease in erythrocytes were noted in birds with avian encephalomyelitis and/or fed sulfaquinoxaline (Morrissette et al., 1959).

Poisoning from the ingestion of lead causes anemia in wild ducks with basophilic stippling of the erythrocytes, according to the findings of Johns (1934). The question of whether or not basophilic stippling of erythrocytes occurs in lead intoxication in chickens is not settled. Key (1924) cites Minot, and Meyer and Speroni to the effect that there is no stippling. Veenendaal (1935) reports having observed anemia without basophilic stippling in chickens with lead poisoning.

Lührs (1936) has described the changes in the blood of pigeons after exposure to high concentrations of war gases (phosgene and mustard). These changes were a relative increase in heterophils and a decrease in lymphocytes. The value for hemoglobin was low immediately preceding death of pigeons exposed to mustard gas. No characteristic degenerative changes of the blood cells were observed.

Under some circumstances of infection or disseminated neoplastic disease there may be a pouring out of unripe blood cells into the blood circulation. This leukemoid reaction may be so marked as to resemble a state of true leukosis. Sometimes there is sufficient evidence of granulomatous or metastatic neoplastic growth in the marrow to explain the reaction by a crowding out of the normal hematopoietic elements

Marked changes occur in the bone marrow and peripheral blood in fowl leukosis. This disease and lymphocytoma are discussed in detail in another section to which the reader is referred for a discussion of the hematology of these diseases.

The functions of the cells of the blood are of such a nature as to make them of great significance in both health and disease. Information on variations of chemical constituents of the blood is accumulating (Sturkie, 1954), and this can be correlated with our information on cells. Knowledge of the changes of the blood and blood-forming organs should consti-

tute an important part of our information on diseases of birds. Such knowledge should consist of more than mere "counts" of the blood cells and should embrace facts concerning the qualitative changes of cells such as indications of degeneration or regeneration of blood cells in the blood and

tissues. Studies should be made at different stages of disease and the changes correlated with the course of the disease. Constant additions to our information are being made in the literature of today and should lead to better understanding of avian hematology in the future.

REFERENCES

Description of cells and hemoglobin in the blood:

Bankowski, R. A.: 1942 Studies of the hemoglobin content of chicken blood and evaluation of methods for its determination. Am. Jour. Vet. Res. 3:573.

Briles, W E: 1960 Blood groups in chickens, their nature and utilization. World's Poultry Sci Jour 16 223.

Bunting, C. H.: 1938. Functions of the leucocytes. In Downey, Hal: Handbook of Hematology. Paul B Hoeber, Inc, New York. Vol I, p. 438

Campbell, C. J., Brown, R. A., and Emmett, A. D. 1914 Influence of crystalline vitamin B, on hematopoiesis in the chick. Jour. Biol. Chem. 152:483.

Chancellor, L., and Glick, B.: 1960. Effect of temperature as a stressor on white blood cells, adrenals and bursa of Fabricius of chicks. Am Jour. Physiol. 198:1346.

Chubb, L. G., and Rowell, J G.: 1959. Counting blood cells of chickens. Jour. of Agr. Sci. 52:263

Cook, S F 1937. A study of the blood picture of poultry and its diagnostic significance. Poultry Sci 16 291.

——, and Harmon, J W. 1933. The regulation of the hemoglobin level in poultry. Am. Jour. Physiol. 105 407.

Denington, E. M., and Lucas A M.: 1955. Blood technics for chickens. Poultry Sci. 34:360.

Diesem, C D., Bletner, J K., and Venske, W. J : 1958. The effect of estradiolcyclopentyl propionate (ECP) on the blood cells of chickens. Avian Dis. 2:63.

——, Venske, W J.; and Moore, E. N.: 1958 The hemogram of healthy chickens. Am. Jour. Vet Res 19(72) 719.

Domm, L V, and Taber, E. 1946 Endocrine factors controlling erythrocyte concentration in the blood of the domestic fowl. Physiol. Zool. 19:258.

Dukes, H H., and Schwarte, L. H.: 1931. The hemoglobin content of the blood of fowls. Am. Jour Physiol. 96 89

Fanguy, Roy C 1961 Blood typing techniques in poultry. Texas Agr. Exp Sta. MP-551-3.

Gordon, A S (Consulting Editor): 1955. Leukocyte functions. Ann. N.Y. Acad. Sci. 59.665.

Harmon, J W, Ogden, E., and Cook, S F. 1932. The reservoir function of the spleen in fowls. Am Jour Physiol 100 99

Hogan, A G., and Parrott, E M 1940 Anemia in chicks caused by a vitamin deficiency. Jour. Biol Chem 132 507.

Hoppe, R. 1935 Beobachtungen uber die Verdauungsleukocytose bei Huhnern. Wiadomości Weterynaryjne 14 41, 1955 Abst in Jahresb. Vet. Med. 58.210.

Jaffe, P 1960 Differences in numbers of erythrocytes between inbred lines of chickens. Nature, London 186 976.

Johnson, E P, and Lange, C J 1939 Blood alterations in typhlohepatitis of turkeys, with notes on the disease Jour. Parasit 25 157.

Jordan, H. E 1938 Comparative hematology. In Downey, Hal: Handbook of Hematology Paul B Hoeber, Inc., New York. Vol. II, p. 700

Kocian, V.: 1936. La composition morphologique du sang des oiseaux suivant les variations, de la pression atmosphérique. Soc de Biol. Compt. Rend. Paris 122:730.

Lucas, A. M., and Jamroz C. 1961 Atlas of avian hematology. U.S.D.A. Washington, D.C Agricultural Monograph 25.

Magrath, T. B., and Higgins, G. M 1934 The blood of the normal duck. Folia Haematol. 51:230.

Melampy, R. M 1948 Cytochemical studies on the chicken erythrocyte. Jour. Biol. Chem. 175:589.

Musheff, C. W. 1956. Cited by Frederickson, T. N., Chute, H. L., and O'Meara, D. C: 1957. Preliminary investigations on the hematology of broiler flocks. Avian Diseases 1:67.

O'Dell, B. L, and Hogan, A C 1943 Additional observations on the chick antianemia vitamin. Jour. Biol. Chem. 149+323.

Olson, C.: 1935. Available methods for examination of the blood of the fowl. Jour. Am. Vet. Med. Assn. 86-474.

——, 1937. Variations in the cells and hemoglobin content in the blood of the normal domestic chicken. Cornell Vet. 27:235.

Palmer, E. L, and Biely, J.: 1935a. Studies of total erythrocyte and leucocyte counts of fowls. I. Repeated erythrocyte and leucocyte counts. Folia Haematol. 53:143.

————: 1935b. II. Effect of 48 hour starvation on total erythrocyte and leukocyte counts. Jour. Am. Vet. Med. Assn. 86:594.

———— (Biely and Palmer): 1935c. III. Variation in number of blood cells of normal fowl. Canad. Jour. Res. Sect. D., Zool. Sci. 13:61.

————: 1935d. IV. Erythrocyte and leucocyte counts of birds raised in confinement. Canad. Jour. Res. Sect. D., Zool. Sci. 13:85.

Riddle, O., and Braucher, P. F.: 1934. Hemoglobin and erythrocyte differences according to sex and season in doves and pigeons. Am. Jour. Physiol. 108 554.

Rostorfer, H. H.: 1919. Comparison of methods for measurement of avian hemoglobin. Jour. Biol. Chem. 180.901.

Scarborough, R. A.: 1931-32. The blood picture of normal laboratory animals. Yale Jour. Biol. and Med. 4: The chicken 202-6; Birds 323-24.

Shaw, A. F. B.: 1933. The leucocytes of the pigeon with special reference to a diurnal rhythm. Jour. Path. and Bacteriol. 37:411.

Sturkie, P. D.; 1913. The reputed reservoir function of the spleen of the domestic fowl Am Jour. Physiol. 138.599.

Tanaka, T., and Rosenberg, M. M.: 1954. Relationship between hemoglobin levels in chickens and certain characters of economic importance. Poultry Sci. 33:821.

Van der Helm, H. J., and Hutsman, T. H. J.: 1958 The two hemoglobin components of the chicken. Science 127:762.

Weiss, N. S., Fisher, H., and Griminger, P.: 1961. Seasonal changes in avian blood pressure related to age, sex, diet, confinement, and breed. Am. Jour. Physiol. 201.655.

Wiseman, B. K.: 1931. An improved direct method for obtaining total white cell counts in avian blood. Proc. Soc. Exper. Biol. and Med. 28.1030.

Young, D. J.: 1937. Erythrocyte counts and hemoglobin concentration in normal female canaries Jour. Parasit. 23·424.

Origins of blood cells and relationships of blood cells in the tissues:

Ackerman, G. A., and Knouff, R. A.: 1959. Lymphocytopoiesis in the bursa of Fabricius Am Jour. Anat. 104:163

Biggs, P. M.: 1957. The association of lymphoid tissues with the lymph vessels of the domestic chicken Gallus domesticus. Acta. Anat. 29:36.

Billingham, R. E., Brent, L., Medawar, P. B., and Sparrow, E M : 1954. Quantitative studies on transplantation immunity. Proc. Roy. Soc. B. 143:. 58.

Bloom, W: 1938 Fibroblasts and macrophages. In Downey, Hal: Handbook of Hematology Paul B. Hoeber, Inc., New York. Vol. II, 1336-73.

————: 1938. Tissue cultures of blood and blood-forming tissues. In Downey, Hal: Handbook of Hematology. Paul B. Hoeber, Inc., New York. Vol. II, 1477-1585.

Burmester, B. R., Severens, J. M., and Roberts. E.: 1941. Blood cells in the bone marrow of the chick before and after hatching. Poultry Sci. 20:391.

Fennell, R. A.: 1947. The relation between age, number, and types of cells in the peripheral circulation of chicken embryos under normal and experimental conditions. Jour. Agr Res. 74:217.

Glick, B: 1956. Normal growth of the bursa of Fabricius in chickens Poultry Sci 35 843.

————: 1957. Experimental modification of the growth of the bursa of Fabricius Poultry Sci 36:18.

————: 1959. The experimental production of the stress picture with cortisone and the effect of penicillin in young chickens. The Ohio Jour. of Sci. 59 (2):81

————, Chang, T. S., and Jaap, R. G.: 1956 The bursa of Fabricius and antibody production. Poultry Sci. 35 224.

Hilgard, H., Burnet, D., and Burnet, F M : 1962. Tolerance as shown by the Simonsen reaction on the chorioallantoic membrane. Australian Jour. Exp Biol and Med Sci 40.233

Huff, C. G., and Bloom, W: 1935. A malarial parasite infecting all blood and blood forming cells of birds. Jour. infect Dis. 57:315.

Jaffé, R. H.: 1938. The reticulo-endothelial system. In Downey, Hal· Handbook of Hematology Paul B. Hoeber, Inc., New York. Vol. II, 974-1271.

Jolly, J.: 1923. Traité Technique d'Hématologie. A. Maloine et Fils. Paris. Vols. 1 and II, p. 1131.

Jordan, H. E.: 1936. The relation of lymphoid tissue to the process of blood production in avian bone marrow Am Jour Anat. 58 249.

————, and Johnson, L P : 1935 Erythrocyte production in the bone marrow of the pigeon Am Jour. Anat. 56-71.

————, and Robeson, J. M: 1942. The production of lymphoid nodules in the bone marrow of the domestic pigeon, following splenectomy. Am Jour. Anat. 71-181.

Mann, F. C. and Higgins, G. M.: 1938. The system of fixed histiocytes in the liver. In Downey, Hal: Handbook of Hematology. Paul B Hoeber, Inc., New York. Vol. II, 1376-1426

Moriba, S., and Nishida, S.: 1956. Hematological and histological changes induced by adreno-corticotropic hormone and formaldehyde stressor in the domestic fowl. Endocrinologia japonica 3 39.

Mueller, A. P., Wolfe, H. R., Meyer, R. K., and Aspinall, R. L.: 1962. Further studies on the role of the bursa of Fabricius in antibody production. Jour. Immunol. 88.354.

Newcomer, E. H., and Connally, J. L.: 1960. The bursa of Fabricius as an indicator of chronic stress in immature chicken. Endocrinology 67:264.

Papermaster, B. W., Friedman, D. I., and Good, R. A.: 1962. Relationship of the bursa of Fabricius to immunologic responsiveness and homograft immunity in the chicken. Proc. Soc. Exper. Biol. and Med. 110:62.

———, and Good, R. A.: 1962. Relative contributions of the thymus and the bursa of Fabricius to the maturation of the lymphoreticular system and immunological potential in the chicken. Nature 196.838.

Ruth, R. F.: 1960. Ontogeny of blood cells. Fed. Proc. 19:579.

———, Allen, C. P., and Wolfe, H. R.: 1962. The effect of thymus on lymphoid tissue. First Conference on the Thymus, University of Minnesota.

Simonsen, M.: 1957. The impact on the developing embryo and newborn animal of adult homologous cells. Acta Path. et Microb. Scand. 40:480.

Szenberg, A., and Warner, N. L.: 1962. Dissociation of immunological responsiveness in fowls with a hormonally arrested development of lymphoid tissues. Nature, London 191:146.

Thorbecke, G. J., Gordon, H. A., Wostman, B., Wagner, M., and Reyniers, J. A.: 1957. Lymphoid tissue and serum gamma globulin in young germfree chickens. Jour. Infect. Dis. 101:237.

The blood in disease:

Buxton, A.: 1960. Pathological changes in the blood of chickens infected with *Salmonella gallinarum*. Jour. of Comp. Path. and Therap. 70:508.

Coles, J. D. W. A.: 1939. Aegyptianellosis of poultry. Proc. Seventh World's Poultry Cong., p. 261.

Cook, F. W. and Dearstyne, R. S.: 1934. Hematology of the fowl. A. Studies on normal avian blood. B. Studies on the hematology of avian typhoid. N.C. Agr. Exp. Sta., Tech. Bul. 44:51.

Cover, M. S., and Meehan, W. J., and Gill, E.: 1955. Studies of hemorrhagic syndromes in chickens. Cornell Vet 45 366:

DeKoch, C.: 1954. Studies on the histopathology and pathogenesis of Newcastle disease of fowls in South Africa, with special reference to the lymphoid tissue. Onderstepoort Jour. Vet. Sci. 26 399.

Dinning, J. S., Meenan, I., Keuh, C. K., and Day, P. L.: 1950. Effects of X-irradiation and urethane treatment on chicken bone marrow enzymes. Proc. Soc. Exper. Biol. and Med. 74 776.

Fantham, H. B.: 1912 Blutbeobachtungen bei Waldhühnern. Deutsch. tierärztl. Worchenschr. 20 247.

Fredrickson, T. N., and Chute, H. L.: 1958. Further studies of chicken blood tests and their application. Avian Dis 2:241.

Gauger, H. G., Greaves, R. E., and Cook, F. W.: 1940. Paratyphoid of pigeons. N.C. Agr. Exp. Sta., Tech. Bul. 62:71.

Goff, S., Russell, W. C., and Taylor, M. W.: 1953. Hematology of the chick in vitamin deficiencies. I. Riboflavin. Poultry Sci. 32.54.

Gray, J. E., Snoeyenbos, G. H., and Reynolds, J. M.: 1954. The hemorrhagic syndrome of chickens. Jour. Am Vet. Med. Assn. 125,144.

Grimal, R.: 1938. Variations de la formule leucocytaire et du rapport lymphomonocytaire dans la tuberculose aigue de la poule. Soc. de Biol. Compt. Rend. Paris 128.655.

Grünınger, P., Fisher, H., Morrison, W. D., Snyder, J. M., and Scott, H. M.: 1953. Factors influencing blood clotting time in the chick. Science 118:379

Hanley, J. E.: 1962. Observations on avian aplastic anemia in Florida. Avian Dis. 6:251.

Ishiguro, H.: 1957. A transmissible anemia (infectious anemia) of the fowl. Bul. Fac. Agr. Yamaguii Univ. No. 8.753.

Jackson, C.: 1936. Incidence and pathology of tumors of domesticated animals in South Africa. Onderstepoort Jour. Vet. Sci and Animal Ind. 6.1.

Jacobi, L.: 1939. Beitrage zur Pathologie der Infektion des Huhnes mit Plasmodium gallinaceum (Brumpt). Arch. f exper. Path u Pharmakol. 191:482.

Johns, F. M.: 1934. A study of punctate stippling as found in the lead poisoning of wild ducks. Jour. Lab. and Clin. Med 19 514.

Johnson, E. P., and Lange, C. J.: 1939 Blood alterations in typhlohepatitis of turkeys, with notes on the disease. Jour. Parasit. 25 157.

Key, J. A.: 1924. Lead studies IV Blood changes in lead poisoning in rabbits with especial reference to stippled cells. Am Jour. Physiol. 70.86.

Kromker, F.: 1937. Ein Beitrag zum Blutbild gesunder und kranker Huhner. Thesis No. 1840, presented to Friedrich-Wilhelms Universty, Berlin. R. Pfau, Berlin, p. 41.

Lubin, G.: 1936. Blutuntersuchungen bei Kampfstoffvergifteten Tauben, zugleich ein Beitrag zur Morphologie des normalen Taubenblutes. Zschr. f. Veterinark 48·129.

Moore, V. A.: 1895-96 Infectious leukemia in fowls—a bacterial disease frequently mistaken for fowl cholera. Twelfth and Thirteenth Ann. Rep. Bur. Animal Ind., U.S.D.A., p. 185.

Morrissette, M. C., McDonald, L. E., and Thayer, R. H.: 1959. Hematology of growing chickens. Poultry Sci. 38:249.

Olson, C.: 1935. The effect of certain ectoparasites on the cellular elements and hemoglobin of the blood of the domestic chicken. Jour. Am. Vet. Med. Assn. 87:559.

———, and Feldman, W. H.: 1936. The cellular elements and hemoglobin in the blood of chickens with spontaneous tuberculosis. Jour. Am. Vet. Med. Assn. 89:26.

———, and Coetchius, G. R.: 1937. The reaction of chickens to certain members of the colon-paratyphoid group of bacteria. Cornell Vet. 27:354.

———, and Levine, P. P.: 1939. A study of the cellular elements and hemoglobin in the blood of chickens experimentally infected with Capillaria columbae (Rud.). Poultry Sci. 18:3.

Olson, N. O., Shelton, D. C., Munro, D. A., and Bletner, R.: 1957. Preliminary blood studies in chickens with a synovitis caused by the infectious synovitis agent, pleuropneumonia-like organisms and a combination of the two agents. Avian Dis. 1:82.

Pomeroy, B. S., and Fenstermacher, R.: 1937. Hemorrhagic enteritis in turkeys. Poultry Sci 16:378.

Rostorfer, H. H., and Rigdon, R. H.: 1946. A physiologic study of hematopoiesis in the duck with malaria. Am. Jour. Clin. Path. 16:518.

Seastone, C. V.: 1935. Pathogenic organisms of the genus Listerella. Jour. Exp. Med. 62,203.

Sharma, F. L., and Seetharaman, C.: 1950. Blood picture in Ranikhet disease of fowls. Indian Jour. Vet. Sci. and Anim Husb. 20,203.

Sturkie, P. D.: 1954. Avian Physiology. Comstock Publishing Associates, Ithaca, New York.

Taylor, W. J.: 1916. A report upon an outbreak of fowl typhoid. Jour. Am. Vet. Med. Assn. 49 35.

Thorp, F., and Graham, R.: 1932. Blood-cell counts in acute avian laryngotracheitis. Jour. Am. Vet. Med. Assn. 80 909.

Veenendaal, H.: 1935. Loodintoxicatie en basophiele korreling der roode bloedlichaampjes. Tijdschr. v. Diergeneesk. 62:244.

Wai, W. Y., and Stafseth, H. J.: 1960. Pullorum disease studies in turkeys. IV. Blood cells and their response to pullorum infection. Poultry Sci. 29:328.

Wannop, C. C.: 1961. The histopathology of turkey "X" disease in Great Britain Avian Dis 5.371.

Ward, A. R.: 1904. Fowl cholera. Calif. Agr. Exp. Sta , Bul. 158

Wickware, A. B.: 1947. The differential blood picture in chickens before and after administration of embryonated eggs of Heterakis gallinae with notes on pathogenicity. Canad. Jour. Comp Med. 11:78.

Wirth, D., and Kubasta, F.: 1939. Studien zur artspezifischen Reaktion der hämatopoetischen Organsysteme (VII, Huhn). Folia Haematol. 62:45.

Yakimoff, W. L., and Rastégaieff, E. F.: 1929. Sur la question des variations cytologiques du sang des poules. Bul Soc. de Path. Exot. 22:766.

———: 1930. Die Spirochätose der Hühner in Nordkaukasus. Zentralbl. f. Bakt I. Originale 117:223.

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5

Principles of Disease Prevention

The trend in all agricultural industries is towards larger units, fewer farmers, and corporate operation. Poultry and turkey industries have been following these trends, and with them have come stress on efficiency of operation and lower cost of production. In the chicken industry, larger laying flocks, a shift to cage layer operation, and mass rearing of broilers are examples. For a number of years turkey flocks have been increasing in size with many small part-time raisers leaving the business. Corporation farming and the tendency towards integration of breeder, hatchery, brooding, rearing, and feed supply operations under one management present new and different problems in all types of management.

The cardinal principles of disease prevention and control, however, have not changed. It will not be the purpose of this chapter to give details on methods adapted to modern trends but only to attempt to outline fundamentals that can be applied to all phases of the industries. The reader

is referred to individual chapters on specific diseases for specific information concerning them. It is also emphasized that to keep abreast with the enormous research now under way, the reader must supplement what is given herein by a constant survey of the current literature. It will be especially important to be on the alert for new information which will be forthcoming as a result of the increased interest in environmental research. Standard textbooks on poultry and turkey production are other sources of information. Examples are Card (1961) and Marsden and Martin (1955).

The same principles of disease prevention apply to poultry as to other livestock; and they are, to a large extent, the same as those applying to human beings. Van Es and Olney (1934) summarize the factors conducive to health and body efficiency: "(1) soundness of body and of constitution and vigor, (2) adequate nutrition, (3) suitable environment, and (4) eradication and control of transmissible

diseases." Although immunity to disease cannot be guaranteed when poultry is reared according to these principles, the grower who observes them will increase his chances of raising a profitable flock.

SOUNDNESS OF BODY AND CONSTITUTION

The most important factor in having a flock of sound, vigorous birds that have good constitutions is the breeding history of the flock. Selection of healthy, well-matured stock will aid in developing a disease-free flock. The ancestry of the breeding stock should be considered; birds from a parent that had some genetic defect, such as a pendulous crop, crooked toes, or a curved spine, should always be avoided. A chick or poult that has come through the season without such a setback will serve much better for propagation than the one that has had several setbacks Before being finally selected as a breeder, each individual should be examined for defects and discarded if abnormal in any way. When buying hatching eggs or day-old chicks or poults, one should demand stock from disease-free breeding flocks that meet the requirements just discussed.

ADEQUATE NUTRITION

An adequate diet supplies all the essentials for normal growth. With any one essential ingredient lacking, or in improper balance, normal development will be retarded. A diseased condition directly or indirectly due to the faulty ration may result. Whether or not many birds die in such cases, slow development may cause as great a monetary loss as if there had been severe mortality. Some of the dietary disorders will be discussed in Chapters 6 and 7.

SUITABLE ENVIRONMENT

The term "environment" refers to the surroundings in which the birds must live Necessarily, this environment varies with the methods of rearing. The obsolete practice of hatching and rearing with hens is an example of an entirely different type of environment from that furnished by the modern method of incubator hatching of eggs and brooder rearing of young birds. Furthermore, the range rearing of poults or chicks is in contrast to the confinement method. In any case, the relation to disease depends on the ability of the environment to aid nature in combatting disease. Dryness, drainage, amount of sunshine, nearness to other species of fowl or other animals on the same premises, location in respect to other farms, type of soil, and shelter facilities are examples of environmental factors that may influence the disease problem.

ERADICATION AND CONTROL OF TRANSMISSIBLE DISEASES

Transmissible diseases, once established, may cause heavy losses. Examples are histomoniasis (blackhead), hexamitiasis, fowl typhoid, fowl cholera, pullorum disease, salmonellosis (paratyphoid), infectious bronchitis, Newcastle disease, chronic respiratory disease (CRD), laryngotracheitis, fowl pox, and coccidiosis. The two general ways of introducing infectious diseases into a flock are by natural and mechanical carriers.

Natural Carriers

The most serious carriers of infections are birds or other animals which have apparently recovered from the disease in question but which still retain the infectious organisms in some part of the body where they continue to multiply and to be eliminated. Among the diseases known to be transmitted by carriers are histomoniasis, hexamitiasis, Leucocytozoon infection, coccidiosis, fowl typhoid, pullorum disease, salmonellosis, tuberculosis, fowl cholera, and many respiratory diseases. Removing carriers from the flock and premises is an effective way of preventing a recurrence of an outbreak. Different methods of accomplishing this end exist; *but one, common to all diseases, is absolute isolation of the adult breeding flock from the growing flock.*

The depopulation method is most ap-

plicable to newly introduced diseases and especially to exotic diseases like fowl plague. It may also be used to rid the premises of diseases such as *tuberculosis*, fowl cholera, fowl typhoid, and infectious laryngotracheitis. Depopulation, as well as other phases of disease eradication, is a community problem and one must have the cooperation of all poultrymen if success is to be insured. The time between depopulation and purchase of disease-free day-old replacement stock will depend on many factors discussed under the diseases in question. Environment plays an important part in the time interval necessary to insure success.

It is extremely important that *all* replacements for any system of management come as pullorum-typhoid-disease free (U.S. Pullorum-Typhoid-Clean) eggs, chicks, or poults. *The purchase of started chicks or poults is an excellent means of introducing many diseases into a flock.* Such diseases as Newcastle disease, coccidiosis, and the various respiratory diseases are especially prone to be spread by purchase of chicks that have been reared from a few days to a few weeks by the hatchery.

The purchase of adult birds for breeding flock replacements is another common way of introducing disease into a flock. All breeding flock owners should therefore always purchase their replacements as hatching eggs or day-old chicks or poults and only from known disease-free sources.

An equal chance is taken by the breeding flock owner who exhibits his breeders at shows or fairs and then returns them to his ranch. If he must show his birds, he should select individuals which can be sold for market purposes after the exhibition is over. Fowl pox, respiratory diseases, and external parasites are likely to be the price paid for returning birds to the home farm following their exhibition at shows. Egg-laying contests fall in the same category as fairs and shows. The increase of cage layer flocks has created an interest in "started pullet" supplier businesses to sell started pullets to cage layer flock owners. This system of obtaining replacements has many advantages, but it can be a dangerous practice as far as disease control is concerned. The system is conducive to the spread of disease and must be very carefully controlled to prevent introduction of new diseases into a flock.

Unfortunately, carriers of the more common diseases of poultry cannot be detected by simplified tests that are practicable. The agglutination tests for carriers of fowl typhoid, for pullorum disease, a few of the other salmonelloses (paratyphoid) and chronic respiratory disease are exceptions. Strict supervision over all supply flocks will help to secure eggs of the best quality.

Chickens may be carriers of many diseases common to both turkeys and chickens. It is equally true that turkeys may be carriers of diseases which may cause severe losses in chickens. Turkeys and chickens can be reared as pen mates or in adjoining yards, provided both species are free from disease; but the chances that chickens may carry blackhead or other diseases or that turkeys may carry chicken diseases are too great to risk (Fig. 5.1). Wild birds, such as quail, pheasants, pigeons, and sparrows, are susceptible to many of the diseases of chickens and turkeys, such as salmonellosis, blackhead, hexamitiasis, respiratory disease, and fowl cholera. Psittacine birds as well as certain wild waterfowl must be considered possible carriers of ornithosis (psittacosis) to turkeys, chickens, and ducks.

Mechanical Carriers

Mechanical carriers include all means by which infectious organisms are accidentally carried from place to place: man, animals, wild birds, insects, dust storms, moving vehicles, feed sacks, poultry crates, and flowing streams.

Man is the worst offender, and the attendant who cares for both an adult and growing flock may be the principal carrier to the young flock. It has been shown that a person may carry viable coccidial oocysts on his shoes for several hours and be responsible for contaminating clean feed in

FIG. 5.1 — Chickens and turkeys should not be reared in the same yard.

the process of mixing it. It has also been proved that coccidial oocysts may remain on the soles of shoes after the wearer has walked for at least one-half mile, and, when washed from the soles, are capable of producing fatal cases of the disease. Thus, if adult turkeys or chickens are to be kept on the same premises with poults or chicks, great care must be taken to prevent spread of the disease from the adults to the young by attendants. This precaution applies also to other diseases.

Visitors, especially other poultry growers, feed salesmen, and service men, are the principal offenders aside from the attendant himself. The poultry grower should avoid visiting neighboring farms if disease is known to be present. Visitors should be cautioned about entering the houses and yards. The feed dealer's or the poultry buyer's truck and the borrowed spray tank that has been making the rounds of the farms may be sources of disease. The used feed sack, the poultry crate that has not been thoroughly cleaned and disinfected after being sent to market, and the hoe or scraper that is used in the pens of the brooder house carriers and then in the brooder house without being cleaned, are other possible sources of disease. The use of paper feed

sacks that can be discarded eliminates the secondhand feed sack problem. Jungherr (1950) has reviewed the literature, and reported his attempts to sanitize used feed bags. He was not successful in finding a satisfactory practical method. The modern tendency towards bulk storage and bulk handling of feed has probably aided in prevention of disease by reduction of the chances of contact with contaminated objects, as well as aiding to eliminate the used feed bag problem.

Since carcasses from diseased birds, offal, and feathers can be classed as possible sources of disease, such material should be burned or buried deep. Contaminated soil, and water polluted by dead birds thrown into streams even at some distance from the premises, are other sources.

Hospitals and hospital yards may be important in spreading disease to different pens or houses on a ranch. Sick birds from several pens congregated in one hospital pen or house and later taken back to their respective quarters may not only carry back the condition for which they were removed but one or more diseases contracted while in the hospital. For this reason hospital pens are not advocated.

Passerine birds, dogs, cats, rodents, and
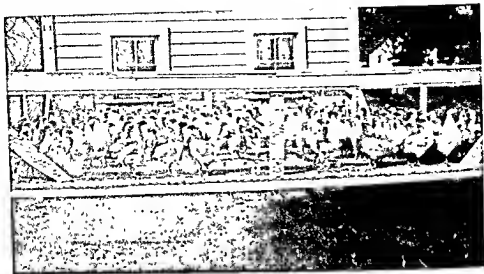
FIG. 5.2 — Wire sun porches are an aid in preventing disease. This shows an example of a sanitary runway attached to the front of a portable brooder house. (Payne, Kans. Agr. Exper. Sta.)

insects are difficult to incriminate as mechanical carriers, but they are possibilities and should be kept away whenever possible.

SANITATION

Sanitation may be defined as the means and measures directed toward establishing and maintaining an environment in which it is safe for animals to exist. The factors considered on the preceding pages are important adjuncts to any sanitary program. Especially important is the elimination of carriers. Other factors to consider are houses, yards, water supplies, and food.

Houses and Yards

The first step in the sanitation of brooder houses is the original construction. Ease of cleaning and disinfection, proper isolation of each unit in the case of the multiple-pen type, separate entrances for each unit, sanitary water and feeding systems, and rodent-proof feed storage containers should be considered when building a brooder house. Facilities for cleaning the individual houses and yards can be arranged by having a gate in the front entrance of each yard.

Wire sun porches and wire platforms

are an aid in preventing disease (Fig. 5.2). It is important that the wire used for sun porches and platforms be heavy enough to hold birds without sagging and that the mesh be of sufficient size to permit droppings to fall through readily. A mesh 1″ x 1″ or 1″ x 1½″ will serve best for all purposes. Even for very young chicks and poults it is seldom necessary to use less than a ½″ x ½″ mesh wire.

The number of birds on a given area, either in the brooder house or on the range, may influence the livability. Overcrowding means more work in keeping the surroundings clean and dry; it also increases the problem of feeding and ventilation. These factors indirectly lower the resistance of birds and facilitate spread of disease. There are many factors to be considered in determining what constitutes overcrowding, but use of common sense by the grower will answer most of the questions as to the number of birds to be housed under various local conditions.

The reader is referred to standard textbooks, current experiment station bulletins, and reliable journals for detailed information on space requirements for chickens and turkeys of various ages.

Yards or ranges should be maintained

free from all infections and infestations. Chickens should never be reared in yards with turkeys. For the confinement method of rearing turkeys, rotation of runs is recommended. In large range areas, rotation of runs is impossible; but feeding grounds and feeding areas can be moved at least twice a week as an aid in preventing accumulations of manure and litter, wherein lies the greatest danger of disease. Good drainage that prevents the formation of stagnant pools in yards is necessary. The probability of introducing disease is directly related to the amount of parasitic invasion. If moisture is not present, only the more resistant organisms can remain alive and infective. Good drainage, such as is found on sandy or gravelly soil, aids in keeping infections at a minimum because of the dilution factor of rains. Dry, hot regions having an abundance of sunshine aid in reducing the possibility of contamination and therefore in preventing disease. The range method, which provides enough ground so that birds can be moved frequently to clean areas, likewise helps.

Water Supply

Since the water supply is no better than the poorest water available, all sources other than those known to be clean and safe from contamination should be removed. The best type of water fountain is of no value in preventing disease if it is allowed to overflow and to form a stagnant pool. The immediate area around the permanent fountain or drinking place should be filled in for several inches with gravel, or the container should be set on a screened platform with a mesh $1'' \times 1''$ or $1'' \times 1\frac{1}{2}''$ to insure a dry area, which will help to prevent the spread of disease. In houses or in yards, wherever possible, an automatic watering system with proper drainage for disposal of the surplus is recommended. Figure 5.3A and B illustrates types of watering devices that are recommended. Many other types of ready-made sanitary water equipment are on the market.

For young birds, from 15 to 20 one-gal-

lon fountains for each 1,000 birds are recommended. Older birds should have from 48 inches to 72 inches of water fountain space accessible on both sides per 1,000 individuals. The number and capacity will vary with conditions, and growers should make sure that there are ample supplies to keep water before the birds at all times.

When automatic systems are used, they should be inspected frequently to insure that they are operating properly. It is especially important to check the pressure systems that are equipped with automatic drug-dispensing devices. Pure, fresh, clean water is the most palatable. If well protected from contamination by body wastes, soil, and feed, it far surpasses the same water which has been medicated for decontamination purposes. Birds do not like most of the drugs recommended for drinking water; often they avoid water because of this dislike. With the continuing increase of use of antibiotics and other drugs as additives to feed and/or water for specific disease control, precautions must be taken to prevent excessive use of them and to insure that the quantities added are in amounts that will be readily accepted by the birds.

Streams and irrigation ditches as a source of water are safe provided they come from uncontaminated sources, are not stagnant, and are flowing at a fair rate of speed. *Pools of stagnant water from overflowing or leaking canals or water from ditches that are not flowing cannot be considered reliable.* Since poisoning from salt water and alkali water has been reported, it may be desirable to have water suspected of having caused poisoning analyzed by a chemist.

Feeds and Feeding Methods

Feed as a mechanical means of carrying infection has already been mentioned. In addition, feed may directly transmit fungus diseases, botulism, and possibly other infections. For these reasons, one should purchase the best feed and protect it from dampness and from all sources of infection. *Moldy feed should never be given.*
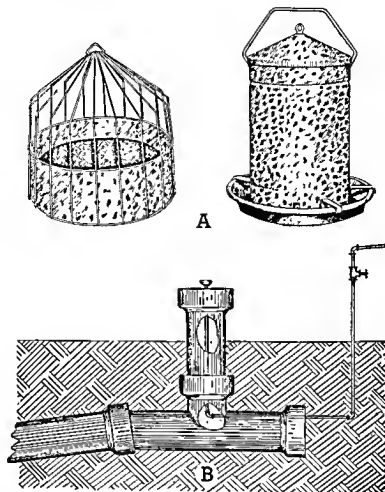
FIG. 5.3 — (A) Two types of commercially made galvanized waterers suitable for poultry. These should be set on wire platforms to insure dry surroundings. (B) "Van Es" type of water fountain. It provides for a continuous flow of water in the bubbler and for passage of overflow into the tile drain. The drinking cup is placed 8 inches above the ground, is kept automatically cleansed, and can be regarded as strictly sanitary. (Van Es, Univ. of Nebr.)

Several outbreaks of mycosis originating from contaminated milk containers have been observed by the author. Failure to wash and scald daily the cans used for transporting milk from the dairy to the range was the most common cause. Improper care of semisolid milk may be another source of mycosis. Forgacs et al. (1962) showed that under some conditions feed contaminated with any one of several species of molds may cause a marked hemorrhagic disease of chickens.

Safeguarding the feed against fecal matter and other refuse by using properly constructed feed hoppers is a necessary procedure in the sanitary program. There are three general types of feeders in common use on poultry farms. These are (1) the mechanical feeder, (2) the trough type, (3) the hanging type. A hanging and trough type are illustrated in Fig. 5.4 A and B. The proper use of any of these types is essential. It is especially important to keep them adjusted properly for the
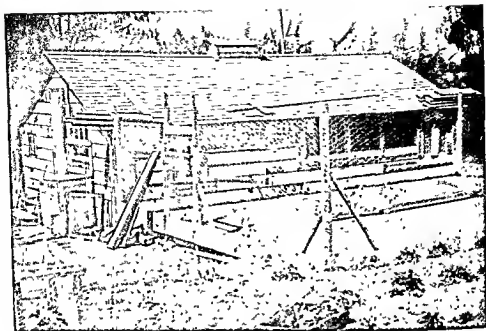
FIG. 5.5 — The type of surroundings that invites rat and mouse invasion and in turn increases disease transmission possibilities.
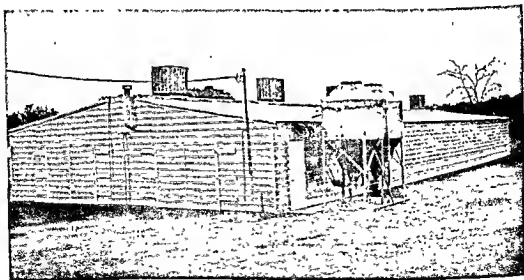
FIG. 5.6 — This modern house has many features which aid in disease prevention. They include outside feed storage tanks, roof ventilation (evaporator coolers), and two large doors on either end to facilitate cleaning between lots. (Pacific Poultryman.)
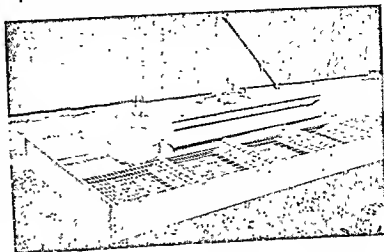
FIG. 5.7—Type of wire platform used at the Los Angeles County (California) Poultry Demonstration Plant, for feeders and waterers. Note the length, width, height, and the size of the wire mesh (1" x 1½"). (Hinshaw, Univ. of Calif. Agr. Exper. Sta., Bul. 613.)

pends on the nature of the environment, the character of the infectious agent to be destroyed, and the method to be used.

Cleaning, before the final application of chemicals, is essential in any disinfection program; cleaning alone will not result in disinfection, but, if carried out properly, it will render disinfection by chemicals more efficient. The following steps are suggested for cleaning and disinfecting:

1. Settle the dust by spraying lightly with the disinfectant to be used. This procedure avoids undue scattering of microorganisms by dust and aerosols.

2. Take all movable equipment out of the building before starting the cleaning operation.

3. Never spread litter or droppings on the land being used for ranging turkeys or chickens. If infection is known to exist, bury or burn the litter.

4. Scrub the walls, floors, and equipment with hot lye solution made by adding 1 pound of lye to 20 gallons of hot water or with a good detergent. An old broom can be used to apply the lye solution; care should be taken to prevent the fluid from getting on the hands and face. About 1 hour after its application, the lye should be rinsed off with hot water.

Used properly, portable high pressure steam cleaning units for cleaning houses and equipment, are of great value in the cleaning and disinfection program. An advantage of using high pressure equipment is that detergents, which aid both in cleaning and decontamination, can be added to the water. Many types are available. (Example: Fig. 5.8.)

5. Spray the walls, floors, and equipment with a good disinfectant of the concentration recommended by the manufacturer. Use a compressed air sprayer for applying the disinfectant, and cover every part of the building or equipment. In modern poultry plants where ventilating ducts present a problem, steam formaldehyde techniques should be considered for decontamination of the duct system.

6. Allow time for drying before using the house and equipment again.

Certain types of hovers and brooderheating equipment are not easily washed and disinfected because of the danger of injuring them. The formaldehyde gas method recommended for disinfecting cabinet types of incubators may be used for such equipment if a gas-proof room is available.

In the early 1950's there was a wave of publicity on the merits of "built-up" or "deep litter" circulated among poultrymen. This was the result of research work stimulated by Kennard (1950) and Kennard and Chamberlin (1950) at the Ohio Agricultural Experiment Station. Moore

FIG. 5.8 — A portable steam cleaner in operation at the Poehlmann Hatchery, Petaluma, California. (S. E. Hall Co., Berkeley, Calif.)

and Chamberlin (1953) suggested "compost litter" as a more appropriate name for "deep litter" and "built-up litter." The data obtained by these investigators indicate that litter, if kept dry, need not be replaced for long periods, and that it need only be replenished from time to time with new litter. This practice is contrary to all previous ideas of proper sanitation, so in spite of the apparent successes in controlled trials, the writer believes that one should proceed with caution when using the compost-litter system.

Since ammonia fumes tend to accumulate as a result of damp litter and may cause a keratoconjunctivitis in chickens (Bullis et al., 1950; and Wright and Frank, 1957), it is important that litter be replaced if excessive ammonia fumes are detected. It is also essential that the litter be kept dry by frequent stirring, especially during the winter months. Birds should be carefully watched for signs of disease that would indicate the system is not working.

Disinfestation

Mechanical or physical means of hindering the development of parasites or destroying them are probably as important as chemical means. Cleaning the yards of all refuse, removing litter and droppings frequently, and constructing the houses so as to prevent the harboring of ticks, lice, and mites are examples of mechanical methods. All methods of fly control — cleanliness, proper care of litter and droppings, and destruction of breeding places — indirectly aid in reducing tapeworm infestation.

The methods recommended for cleaning and disinfection are also applicable in the disinfestation program. Yards are best treated by frequent cleaning and by rotation. The former dilutes the amount of infection or infestation and allows the sun better opportunity to exert its influence on the remaining parasites. Rotation of runs at regular intervals allows the sun and the other natural elements to free a given area of parasites. No satisfactory cheap disin-

festant for the soil has been found.

Plowing of the yards is not recommended unless necessary for weed control or unless a crop-rotation system is combined with the poultry-rearing program. Plowed yards soon become dusty, tend to become pitted with holes that collect water during rains, and are harder to clean than yards that are left unplowed.

DISINFECTANTS

The number of chemicals sold as disinfectants is great. Some are worthless; others are excellent disinfectants but have undesirable characteristics. Among the properties of an ideal disinfectant are (1) low cost per unit of disinfecting value, (2) ready solubility in hard water, (3) relative safety to man and animals, (4) efficient deodorization, (5) easy availability, (6) nondestructibility to utensils and fabrics, (7) stability when exposed to air, (8) absence or minimum of objectionable and lingering odor, (9) effectiveness for a large variety of infectious agents. Obviously, no one chemical will have all these properties; but the list will serve as a guide.

Many disinfectants of similar compositions are sold under different trade names. Before buying a product under an unfamiliar trade name, one should compare types and values with a well-known product. The directions for dilution given by the manufacturer should be followed in making up a disinfectant for use. These directions are usually based on the concentration of the product; and by comparing the dilution factor of two disinfectants that have other properties being equal, one can determine the relative cost of the two. For additional information and references on disinfectants and their use the reader is referred to Reddish (1957), Phillips and Warshowsky (1958), and Glick et al. (1961), as well as to textbooks on pharmacology and therapeutics.

Phenol, or Carbolic Acid

Phenol is a chemical substance obtained from coal tar. In its pure form it occurs as colorless needles having a characteristic odor familiar to everyone. It is usually sold in water solutions and is too expensive for general use. This is the chemical used as a basis for determining the phenol coefficients of disinfectants. For a complete discussion of the phenolic compounds the reader is referred to Klarmann and Wright (1957).

Cresols

Cresols are thick yellow or brown liquids, miscible with water but only slightly soluble. They form the bases for a large number of the commercial brands of disinfectants, made by combining cresol with a soap base.

A list of cresylic disinfectants which are permitted for use for official disinfection is published periodically by the United States Department of Agriculture, Agricultural Research Service, Animal Disease Eradication Division. This list (Anderson, 1961) will serve as a guide for the use of specific products.

Pine Oil

Pine oil has proved satisfactory as a disinfectant and has the advantage of being less injurious to the skin than the cresol compounds. The odor is also less objectionable. It is especially well suited for use on floors of public buildings and could be used to advantage in hatcheries, feed stores, etc. A good commercial pine oil disinfectant should contain at least 80 per cent high quality pine oil.

Hypochlorites

Chlorine is the basis of the disinfectants known as hypochlorites. Hypochlorites, according to Hadfield (1957), are available as powders containing calcium hypochlorite and sodium hypochlorite combined with hydrated trisodium phosphate and as liquids containing sodium hypochlorite.

The products containing sodium hypochlorite are essentially liquids ranging in concentrations from 1 to 15 per cent. The

15 per cent solutions are used to prepare 5 per cent solutions with water for bleaches and sanitizing agents. Germicidal potency of hypochlorites is dependent upon both the concentration of available chlorine and the pH of the solution, or upon the amount of hypochlorous acid formed which is dependent upon both these factors. The influence of pH, especially in dilute solutions, is even greater than the per cent of available chlorine. If used according to directions, hypochlorites are highly efficient. Their principal use is for disinfecting limited areas such as incubators, small brooders, and water and feed containers. *All surfaces to be disinfected with hypochlorite solutions must first be thoroughly cleaned in order to insure the greatest efficiency. Stock supplies should be kept in dark, cool places, and the containers should be tightly sealed when not in use.*

Chlorinated Lime

Chlorinated lime, known as bleaching powder, is prepared by saturating slaked lime with chlorine gas. It should contain from 30 to 35 per cent of available chlorine The U.S.D.A. Animal Disease Eradication Division recognizes chlorinated lime containing at least 30 per cent available chlorine for official disinfection when used in proportions of 1 pound to 3 gallons of water. Chlorinated lime has been largely supplanted by calcium hypochlorite products containing 70 per cent available chlorine. These products are used for chlorination of water supplies, swimming pools, sewage effluents, and for preparation of bleaching, and sanitizing solutions. Fresh solutions must be prepared daily.

All products containing chlorine must be handled with care because free chlorine is destructive to fabrics, leather, and metal.

Quicklime (Unslaked Lime, Calcium Oxide)

The action of quicklime depends on the liberation of heat and oxygen when the chemical comes in contact with water. On the poultry farm its use is limited to small yard areas that are damp and cannot be exposed to the sun, to the disinfection of drains and fecal matter, and to whitewashes. Adding chlorinated lime to quicklime at the rate of 1 pound to 40 gallons of wash increases its disinfecting value in whitewashes. As quicklime has a caustic action, birds should be kept away from it until it has become thoroughly dry.

Hydrated lime, according to Yushok and Bear (1944), when used as a preservative and deodorizing agent for poultry manure also has value as a partial disinfectant. Mixed with fresh manure at the rate of 200 pounds per ton of manure, it was found to have a bactericidal effect on *Salmonella pullorum, Salmonella typhimurium, Salmonella gallinarum,* and *Pasteurella multocida* in a 15-minute period. Similarly it prevented the sporulation of coccidial oocysts and the segmentation and embryonation of *Ascaridia galli* eggs. Another advantage of this use pointed out by them is that the treated manure is unattractive to flies and rodents. Fly maggots are not produced in the treated dropping pits, and both mice and rats avoid them.

Lye

Lye is an excellent cleansing agent, valuable in any disinfecting program. A 2 per cent solution of sodium hydroxide (soda lye) is a good disinfectant for many of the pathogenic microorganisms.

Formaldehyde

Formaldehyde is a gas, sold commercially in a 40 per cent solution (37 per cent by weight) with water under the name of formalin. For spraying it is used in a 10 per cent solution of formalin (that is, a 4 per cent solution of formaldehyde). It may also be purchased as a powder known as paraformaldehyde (paraform, triformal). When heated this powder liberates formaldehyde gas, and if used in proper portions may be substituted for formalin as a source of the gas. Manufacturer's directions on amounts to use for each type of equipment and the means of

liberating the gas must be carefully observed (see Fig. 5.10).

Though a powerful disinfectant, form-aldehyde has many disadvantages, especially its volatility, penetrating odor, caustic action, and tendency to harden the skin — properties which make it disagreeable to apply. Its chief advantages are as follows: (1) it can be used as a gas or vapor for fumigation of incubators or small rooms; (2) it is relatively nontoxic to animals and fowls; (3) it is an efficient disinfectant in the presence of organic matter; and (4) it does not injure utensils and spraying equipment with which it comes in contact.

Its use on the turkey or chicken farm is limited to disinfection of brooder equipment, incubators, water and feed containers, and occasionally — during outbreaks — fumigation of clothing and small utensils that are difficult to disinfect by other means. Fumigation of brooder houses with formaldehyde is, as a rule, impractical because of the difficulty in making them airtight.

Fumigation of incubators, incubator rooms, and holding rooms is a practical procedure. Most incubator manufacturers have recommendations for their type of machine; and their directions should be followed.

The National Poultry and Turkey Improvement Plans (1963) describe in detail the official recommendations for hatchery sanitation including incubator fumigation. When fumigating a room or an incubator, one must have the space airtight and the room temperature and humidity as high as possible. For most efficient disinfection of incubators, Bushnell and Payne (1931) recommend a wet-bulb thermometer reading of 85° to 95° F. Disinfection is uncertain in rooms having a temperature of less than 70° and a relative humidity of less than 70 per cent.

Formaldehyde gas may be liberated from formalin-soaked cheesecloth by the technique described by Graham and Michael (1933). When this method is used the incubator must be thoroughly dry-cleaned. Approximately 20 cc. of formalin is then used for each 100 cubic feet of incubator space. A series of saturated cloths with total area enough to carry the formalin without dripping is suspended under or near the circulating fans, and left until the formalin has completely evaporated.

Formaldehyde gas is most often generated by mixing formalin (40 per cent formaldehyde) and potassium permanganate. For routine purposes 35 cc. (1.2 ounces) of commercial formalin and 17.5 grams (0.6 ounce) of potassium permanganate for each 100 cubic feet of incubator space are mixed together in an earthenware or enamelware vessel having a volume of four to five times the amount of material used. The vessel should be placed above the floor in the middle compartment of the incubator (Fig. 5.9). The doors should be kept closed for at least 10 minutes to allow the gas to penetrate to all parts of the machine. Equipment for generating and introducing the gas through the intake parts of certain types of machines is obtainable from the manufacturers.

Insko et al. (1941) and Insko (1949) recommend using from two to three times the normal amount of potassium permanganate and formalin between hatches when omphalitis is being transmitted in an incubator. Figure 5.10 illustrates one method of heating paraformaldehyde powder to liberate formaldehyde gas. This electric pan should be equipped with a thermostat and a timer which can be controlled from outside the incubator or fumigation chamber.

Formaldehyde can be used successfully when eggs are in the incubator. Insko et al. (1941) and Insko (1949) warn against fumigation during the first three days of incubation because the embryos are then most susceptible to formaldehyde. Fumigation with formaldehyde destroys or attenuates the pathogenic organisms in the incubator, but not within the egg nor within the body of the hatching chick or poult. Its principal use, therefore, is in

FIG. 5.9 — A method of fumigating an incubator with potassium permanganate and formalin. (Graham and Michael, Univ. of Ill., 1933.)

FIG. 5.10 — An electrically heated pan for use in liberating formaldehyde gas from paraformaldehyde. (Vineland Poultry Laboratories photo.)

disinfecting incubators between hatches and, in some instances, during the early stages of a hatch. Before fumigation of incubators during the hatching period, advice should be sought from one familiar with the procedure to determine the possible need and methods. *In general, fumigation of hatching chicks or poults is not recommended.* Precincubation fumigation of hatching eggs as an aid in prevention of salmonellosis is described by Stover (1960). The method used by him is essentially that used for incubator fumigation.

The use of formaldehyde, as well as other gaseous sterilization techniques, is discussed in detail by Phillips (1957) and by Glick *et al.* (1961). A method, referred to by them, which is used for decontamination of large enclosed areas, could be adapted for use by the poultry industry, especially for use in ventilating systems of large broiler-producing plants. It consists of liberation of formaldehyde gas by steam. In this system, commercial grade formaldehyde solution (37 per cent) with 12 per cent methanol (5 parts 37 per cent formaldehyde, and 3 parts methyl alcohol adjusted to pH 5.0) is liberated into the space by a steam ejector. One ml. of formaldehyde solution is disseminated for each cubic foot of air flow for 30 minutes; i.e., if the air flow is 600 c.f.m., then (600 x 1 x 30) 18,000 ml. of formaldehyde solution will be disseminated in 30 minutes. The temperature of the space to be decontaminated must be maintained at above 70° F. and the relative humidity above 70 per cent. Proper temperature and humidity may be assured by allowing the steam ejector to operate for a few minutes before adding the formaldehyde solution. The purpose of adding methanol to the formaldehyde is to reduce deposits of polymers on interior surfaces of the treated spaces.

For smaller space areas such as cabinets, small rooms, and incubators, a portable vaporizer such as the Hydro-mist Vaporizer, Model H, shown in Fig. 5.11 may be used to disperse the formaldehyde gas.[1] This and other types commonly used for decontamination of air filtration systems are described by Decker *et al.* (1962).

Ethylene Oxide

Ethylene oxide is a colorless gas at ordinary temperatures, liquefying readily at 10.8° C. and freezing at –111.3° C. The liquid is miscible with water and all organic solvents in all proportions. It is highly flammable and for this reason is dangerous to use by itself. It is now extensively used in the form of a low-pressure mixture with nonflammable chlorofluorohydrocarbons (Freons) in a disposable 16-oz. can (Haenni *et al.*, 1959). Chambers for utilization of such mixtures are described by Schley *et al.* (1960) and Glick *et al.* (1961).

It probably has limited use on poultry farms, hatcheries, or processing plants, because of its flammability and need for dispensing under slight pressure. It does, however, have many advantages over formaldehyde gas and has been shown to be effective against many poultry pathogens including viruses. Mathews and Hofstad (1953) showed that ethylene oxide was effective against 15 animal viruses including the viruses of infectious laryngotracheitis, fowl pox, infectious bronchitis, and Newcastle disease. Lorenz *et al.* (1950) used it successfully to prevent penetration of *Pseudomonas* sp. through the shells of eggs.

The penetrating properties are excellent, and for this reason it might prove of value for sterilizing litter, feed, and certain equipment. Unlike formaldehyde, ethylene oxide gas is more reactive at lower relative humidities. Phillips (1957) states that the advantages of ethylene oxide sterilization are not in the speed, simplicity, or inexpensiveness of the treatment, but rather in the fact that many types of materials can

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[1] The technique for steam formaldehyde sterilization was furnished through the courtesy of Mr. Everett Hanel, Safety Division, Fort Detrick, Maryland.

FIG. 5.11 — A steam-pressure vaporizer as illustrated can be used for dispersing formaldehyde gas for decontaminating small space areas. (U. S. Army photo.)
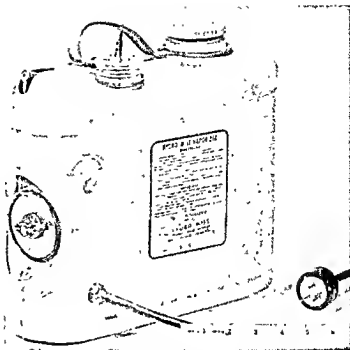
be sterilized with the least damage to the material. For a complete review on this method of disinfection the reader is referred to Phillips' report.

Beta-Propiolactone (BPL)

Beta-propiolactone (BPL) is a newcomer in the field of vapor disinfectants (Hoffman and Warshowsky, 1958; and Glick et al., 1961). BPL more nearly resembles formaldehyde than it does ethylene oxide in its germicidal properties. According to Hoffman and Warshowsky, it possesses a number of advantages over formaldehyde as a gaseous sterilant, especially with respect to increased antimicrobial activity and lessened persistency. It does not polymerize readily on surfaces so leaves little or no residue. In a liquid state it is more toxic than formaldehyde and care must be taken to avoid contact with the skin.

Vaporizers suitable for dissemination of formaldehyde are suitable for BPL (Fig. 5.11). One gallon of lactone is vaporized for each 12,000 cubic feet of space (Spiner and Hoffman, 1960).

It acts more rapidly, but can be used under the same conditions as formaldehyde. Rooms do not need to be hermetically sealed but doors and windows should be closed. After a hold period of 2–3 hours, doors and windows may be opened and forced ventilation applied. It is necessary to enter a room or building only with protective clothing and respiratory protection until proper airing of 2–3 hours has been allowed.

Copper Sulfate (Bluestone)

Although copper sulfate and other salts of copper have a marked toxic effect upon some of the lower forms of life, they are not considered good general disinfectants. Copper sulfate is effective against algae and certain fungi and may prove of some value in outbreaks of fungus diseases. Copper sulfate of a greater concentration than 1 part in 500 of water may be toxic when given as the only source of drinking water. Turkeys do not like copper sulfate solutions of any concentration and will seek other water supplies if they are available.

A 1:2,000 concentration of copper sulfate in drinking water will be consumed readily, if no other drinking water is available. A 0.5 per cent solution may be of value for disinfecting feed hoppers, water fountains, and areas around these in fungous-disease outbreaks.

Mercuric Chloride (Bichloride of Mercury, Corrosive Sublimate)

Although a powerful disinfectant, mercuric chloride is limited in usefulness by its cost, toxicity, and marked corrosive action on metals. It is commonly used in 1:1,000 dilution with water. Because its value is markedly lowered by the presence of organic matter and because it has certain other undesirable properties, it cannot be recommended for disinfection of litter or houses.

Quaternary Ammonium Compounds

Within the past few years much publicity has been given to the disinfecting value of quaternary ammonium salts. There are a number of these products now on the market, and they are generally considered to be good disinfectants if used according to directions. They are water clear, odorless, nonirritating to the skin, good deodorants, and have a marked detergent action. These compounds are recommended especially for disinfection of eggs and for general use around the hatchery. It is important to remember that quaternary ammonium compounds cannot be used in soapy solutions. It is also important that all surfaces to be disinfected be thoroughly rinsed with water to remove any residue of soap or anionic detergent before using a "quat" for sterilizing purposes. An excellent reference on quaternary ammonium compounds is Lawrence (1957).

Sunlight and Ultraviolet Radiation

The sun's direct rays are the best disinfectant known. Since, however, the material to be treated must be in thin layers and exposed to the direct rays, this method of disinfection is limited to yards and to utensils that can be thoroughly cleaned before being exposed. The construction of most poultry houses prevents efficient disinfection by the sun. A cement platform fully exposed to the sun makes a convenient place for treating movable equipment. If properly constructed with a drain, such a platform can be utilized as a washing and disinfection rack.

There are many types of germicidal (ultraviolet) lamps now being advertised for use on the poultry farm and in the hatchery, but there is not enough scientific evidence available to warrant a recommendation for their general use. For a complete review on the use of UV radiation in microbiological laboratories the reader is referred to Phillips and Hanel (1960).

Hot Water

Hot water adds to the efficiency of any disinfectant and, if applied in the form of boiling water or live steam, is effective without the addition of any chemical. Detergents added to systems for generating and disseminating hot water and steam will increase cleaning and decontaminating efficiency. Live steam must be applied directly to the part to be disinfected (see Fig. 5.8).

Dry Heat

Dry heat in the form of a flame is effective provided the flame comes into contact with the bacteria to be killed. According to experiments by Stafseth and Camargo (1935), the fire guns commonly used on poultry farms are not highly efficient as a means of disinfection. *All methods involving direct flame are dangerous fire hazards.*

Central heating systems, construction of poultry houses to utilize solar heat, and these combined with air conditioning and proper humidity control are trends of modern poultry management research. Agricultural engineers are currently doing much research on these problems in attempts to control moisture in poultry houses. Application of principles being de-

veloped will have a marked effect on future sanitation recommendations. The reader is urged to keep abreast with such developments in order to be in a position to make use of them in disease prevention programs.

DISINFESTANTS

Disinfestants, sometimes called parasiticides, destroy animal parasites such as lice, mites, ticks, and fleas. Their use is recommended only as an adjunct to a properly conducted sanitary control program. Many disinfestants are also destructive to lice, mites, and other similar parasites, provided they come in contact with the parasite. Many, however, are useless as disinfestants.

The possible hazards to man and other animals from use of many of the modern pesticides must always be remembered when considering their use. A recent review reference on this subject is Rogoff (1961).

Crude Oil, Distillates, and Similar Cheap Oils

Petroleum oils are excellent and cheap agents for the destruction of lice, mites, and ticks but have been largely replaced by the efficient and easier to apply, more recently developed insecticides (DDT, lindane, malathion, etc.).

Bullis and Van Roekel (1944) have reported that exposure of chicks to the fumes of coal-tar creosote oil, anthracene oil, and certain mite paints too soon after use in a brooder house may cause anasarca (ascites, watery belly).

Nicotine Sulfate

A 40 per cent solution of nicotine sulfate, such as is sold under the trade name of Black Leaf 40, once in general use for controlling lice, has been largely replaced by newer pesticides. Its action depends on a volatile substance that penetrates the feathers of the birds when it is painted on the perches just before they go to roost. The method is not well adapted to control of lice on turkeys under rearing conditions

where the perches are usually placed out of doors.

Sodium Fluoride

This, either as a dust or as a dip, is effective for ridding birds of lice. The dusting method is probably the most desirable. It consists of rubbing a pinch or two of the powder into the parts most often infested with lice (on the tail, under the wings, on the neck and head, and on the breast). As in the case of nicotine sulfate, it has been largely replaced by newer pesticides.

DDT (Dichloro-diphenyl-trichloroethane)

Certain precautions must be given in the use of this much-publicized insecticide. In areas where DDT has been used extensively in fly control, resistant strains of flies have developed to such an extent as to render DDT almost valueless for their control. Because of widespread development of DDT-resistant species of all insects, it has been largely replaced by more recently developed insecticides discussed below. The hazards in its use have also been proved to be greater than originally thought. When DDT is used on farms, wettable powders are probably the most usable type. DDT is effective against mosquitos, the fowl tick, and the black fly (Simulium), provided susceptible strains exist, but is no more effective against lice than sodium fluoride.

Malathion (O, O-dimethyl dithiophosphate of diethyl mercaptosuccinate)

This drug is effective against lice, mites, and flies, and may be used as a spray in a 0.5 per cent emulsion or as dust when applicable. The U.S. Food and Drug Administration has accepted it for direct application as dust and has set a residue tolerance of 4 parts per million as the amount which can be safely found in meat. They require a zero tolerance in eggs. Furman and Coates (1957) found it effective against the Northern fowl mite

FIG. 5.12 — Use of Burdizza forceps in killing a turkey. When brought into a closed position carefully the jaws separate the vertebrae and sever the spinal cord and jugular vein without breaking the skin. (Hinshaw, Univ. of Calif.)

shedding of blood and thus prevent the spread of infections that are present in the blood stream. A convenient tool for the purpose is a Burdizza forceps like that used for castrating calves (Fig. 5.12). Another means of killing birds for necropsy is by electrocution.

4. Burn or bury dead birds. If buried, they should be placed deep enough to insure their not being dug up by dogs or other animals. See section on Disinfection for recommendations on disposal of droppings, and use of litter for disinfection procedures.

A disposal pit such as is illustrated in Fig. 5.13 is superior to incineration as conducted on most poultry farms and by hatcheries. Such pits are easily and cheaply constructed and are efficient. The roof of such pits and especially the "manhole" covering must be airtight to prevent escape of odors and avoid the attraction of flies. Periodic spraying of the roof of the pit with an insecticide is suggested. Open disposal pits are not recommended.

An electrically heated septic tank for disposal of dead birds and waste products on large poultry farms and processing plants has been developed by U.S.D.A., A.R.S., in cooperation with the Maine and Connecticut Agricultural Experiment Stations (Agriculture Research 5(9):14, Mar., 1957). The method consists of digesting

the carcasses and/or waste products in a heated septic tank. A 500 gallon tank will meet the needs of a 10,000 to 20,000 broiler farm. Heat is applied at 100° F. and requires 2 to 3 kilowatt hours of electricity per day to maintain this daily temperature
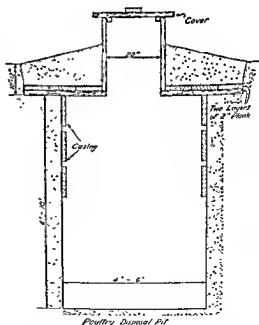
FIG. 5.13 — Poultry disposal pit. Such a pit can be made any size that is convenient, and is valuable for disposal of hatchery wastes as well as carcasses. (Hinshaw, Univ. of Calif.)

for the two weeks needed for destruction of all but the bones of carcasses. The system depends on mesophilic bacteria which multiply best at 90° to 100° F. to accelerate decomposition. Neutralizing the mass at intervals with lime and adding hot water further accelerates the action and speed of decomposition.

5. Thoroughly clean and disinfect all houses and equipment. If the affected birds are in yards, these should be cleaned of all refuse to allow the sun to aid in disinfecting all parts.

6. Keep fresh water before the birds at all times. The water containers should be thoroughly washed and disinfected at least once daily. If medicated water is necessary, it is essential that drugs which are specific for the disease are used and that they are not distasteful to the birds. Birds do have the ability to taste and will seek other sources or go without water if it is distasteful. It is also essential to have enough waterers available so that the birds do not have to walk more than 8 to 10 feet to get a drink. If automatic medicators are installed in watering systems that depend on gravity for delivery to the birds, it is necessary that the elevation is great enough to insure delivery of proper mixtures to all the fountains. Stagnant pools or irrigation ditches should be fenced off so the birds cannot use water from them.

7. Clean and disinfect thoroughly all feed hoppers daily.

8. Thoroughly inspect the food to determine the possible presence of decayed fish or meat scraps, spoiled milk, moldy grain, poisonous weeds, or other sources of possible trouble.

9. Avoid sudden changes of feed. If the feed is the cause of the trouble, a new diet is warranted; but any changes should be made by gradually increasing the new formula.

10. The convalescent stage of any disease is the most important one. Poults or chicks die from lack of feed and water in a very short time. Getting them to eat after they have been ill, even for a short time, is often a very difficult task. It must be accomplished, however, if the mortality is to be reduced to a minimum. Feeding small quantities of mash at frequent intervals often aids in restoring feeding habits.

Here again is emphasized the importance of having enough waterers and feeders so that a bird does not have to walk over 8 to 10 feet to eat or drink (Fig. 5.14). Waterers need to be in close proximity to feeders. Often convalescing birds can be encouraged to eat and/or drink by frequent visits to the pen or house by an attendant. He should not only look into the house, but should wander among the birds, adjust waterers, stir the feed in hoppers, or add small amounts of feed. All these tasks cause birds to become active and eat at least small quantities.
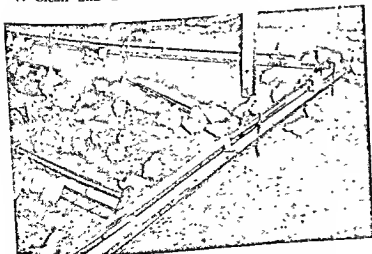
FIG. 5.14 — It is essential to have plenty of feeders and waterers in close proximity to each other. (Everybody's Poultry Magazine.)

REFERENCES

Anderson, R. J.: 1961. Cresylic disinfectants permitted for use in official disinfection. Revised list July 1961. U.S.D.A., Anim. Dis. Erad. Div. Memo 510.10.

Bullis, K. L., Snoeyenbos, G. H., and Van Roekel, H.: 1950. A keratoconjunctivitis in chickens. Poultry Sci. 29:386.

———, and Van Roekel, H.: 1944. Uncommon pathological conditions in chickens and turkeys. Cornell Vet. 34:312.

Bushnell, L. D., and Payne, L. F.: 1931. Dissemination of pullorum disease in the incubator. Kans. Agr. Exper. Sta., Tech. Bul. 29.

Card, L. E.: 1961. Poultry Production. 9th ed. Lea and Febiger, Philadelphia, 409 pages.

Decker, H. M., Buchanan, L. M., Hall, L. B., and Goddard, K. R.: 1962. Air filtration of microbial particles. U.S. Pub. Health Service Publ. No. 953:1.

Forgacs, J., Koch, H., Carll, W. T., and White-Stevens, R. H.: 1962. Mycotoxicoses. 1. Relationship of toxic fungi to moldy-feed toxicosis in poultry. Avian Dis. 6:363.

Furman, D. P., and Coates, W. S.: 1957. Northern fowl mite control with malathion. Poultry Sci. 36:252.

Glick, C. A., Gremillion, G. G., and Bodmer, G. A.: 1961. Practical methods and problems of steam and chemical sterilization. Proc. Animal Care Panel 11:37.

Graham, R., and Michael, V. M.: 1933. Incubator hygiene in the control of pullorum disease. Ill. Agr. Exper. Sta., Cir. 403.

Hadfield, W. A.: 1957. Chlorine and chlorine compounds. Chapter 23 in Reddish, G. F., Antiseptics, Disinfectants, Fungicides, and Sterilization. 2nd ed., Lea and Febiger, Philadelphia.

Haenni, E. O., Affens, W. A., Lento, H. G., Yeomans, A. H., and Fulton, R. A.: 1959. New nonflammable formulations for sterilizing sensitive materials. Indust. & Eng. Chem. 51:685.

Hoffman, R. K., and Warshowsky, B.: 1958. Beta-propiolactone vapor as a disinfectant. Applied Microbiol. 6:358.

Insko, W. M., Jr.: 1949. Physical conditions in incubation. Chapter 6 in Taylor, L. W., Fertility and Hatchability of Chicken and Turkey Eggs. John Wiley and Sons, Inc., New York.

———, Steele, D. G., and Hinton, C. M.: 1941. Effect of formaldehyde fumigation on the mortality of chick embryos. Ky. Agr. Exper. Sta., Bul. 416:117.

Jungherr, E.: 1950. Studies on sanitizing used feed bags. Jour. Am. Vet. Med. Assn. 117:324.

Kennard, D. C.: 1950. Floor litter management as a factor in poultry nutrition. World's Poultry Sci. Jour. 6 177

———, and Chamberlin, V. D.: 1950. Mortality of chicks as affected by floor litter. World's Poultry Sci. Jour. 6:183

Klarmann, E. G., and Wright, E. S.: 1957. Phenolic compounds. Chapter 22 in Reddish, G. F., Antiseptics, Disinfectants, Fungicides, and Sterilization. 2nd ed., Lea and Febiger, Philadelphia

Lawrence, C. A.: 1957. Quaternary ammonium compounds. Chapter 24 in Reddish, G. F., Antiseptics, Disinfectants, Fungicides, and Sterilization. 2nd ed., Lea and Febiger, Philadelphia.

Lorenz, F. W., Starr, P. B., and Bouthilet, R.: 1950. Fumigation of shell eggs with ethylene oxide. Poultry Sci. 29 545.

Marsden, S. J., and Martin, J. H.: 1955. Turkey Management. 6th ed., The Interstate Co., Danville, Ill. 999 pages

Mathews, J., and Hofstad, M. S.: 1953. The inactivation of certain animal viruses by ethylene oxide (Carboxide). Cornell Vet. 43.452

Moore, E. N., and Chamberlin, V. D.: 1953 Compost litter. Nufaid News 31(Sept):18.

National Poultry and Turkey Improvement Plans 1963. Subpart D—Sanitation procedures. U.S.D.A. Misc. Publ. 739 35.

Phillips, G. B.: 1957 Gaseous sterilization. Chapter 30 in Reddish, G. F., Antiseptics, Disinfectants, Fungicides, and Sterilization 2nd ed., Lea and Febiger, Philadelphia.

———, and Warshowsky, B 1958. Chemical disinfectants. Ann. Rev. Microbiol. 12.525.

Phillips, G. B., and Hanel, E.: 1960 Use of ultraviolet radiation in microbiological laboratories. U.S. Library of Congress, P. B. 147 043. Listed in US Govt. Res Reports, 34(2), Aug. 19, 1960 P 122

Reddish, G. F. (editor) 1957. Antiseptics, Disinfectants, Fungicides and Sterilization. 2nd. ed., Lea and Febiger, Philadelphia. 975 pages.

Rogoff, W. M.: 1961. Chemical control of insect pests of domestic animals In Advances in Pest Control Research. Interscience Publishers, Inc., New York 4.155.

Schley, D. G., Hoffman, R. K., and Phillips, G. R.: 1960. Simple improvised chambers for gas sterilization with ethylene oxide. Applied Microbiol. 8 15.

Silver, J., Crouch, W. E., and Betts, M. E.: 1942. Rat proofing buildings and premises. U.S.D I. Conservation Bul. 19.

Spiner, D. R., and Hoffman, R. K.: 1960. Method for disinfecting large enclosures with B propiolactone vapor. Appl. Microbiol. 8:152

Stafseth, H. J., and Camargo, F.: 1935. On the disinfection of poultry houses by means of fireguns. Jour. Am. Vet. Med. Assn. 86:162.

Storer, T. I.: 1960. Controlling rats and mice. Calif. Agr. Exper. Sta. Ext. Leaflet 127.

———, and Mann, M. P.: 1946. Bibliography of Rodent Control. OSRD, Committee on Med. Res., NRC Insect Control Comm. Rep. 182-521 + 57 pp. National Res. Council, Washington, D.C.

Stover, D. E.: 1960. Fumigation of hatching eggs. Calif. Dept. Agr. Bul. 49.30

Van Es, L., and Olney, J. F.: 1934. Diseases of poultry—their nature and control. Nebr. Agr. Exper. Sta., Bul. 290.

Wright, G. W., and Frank, J. F.: 1957. Ocular lesions in chickens caused by ammonia fumes. Canad. Jour. Comp. Med. 21:225.

Yushok, W., and Bear, F. E.: 1944. Poultry manure, its preservation, deodorization and disinfection, N.J. Agr. Exper. Sta., Bul. 707.

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Proteins, Carbohydrates, Fats, Fiber, Minerals, and Water in Poultry Feeding

In order to maintain poultry in good physical condition and to obtain normal growth, egg production, and hatchability, rations must be fed that are adequate in all nutritive essentials. Whenever a serious deficiency of any one of these essential substances occurs, symptoms of deficiency develop which in some instances are characteristic. These are frequently preceded and accompanied by nonspecific symptoms such as retarded, uneven growth; rough feather development; decreased egg production; and lowered hatchability. When the deficiency is only a partial one, these may be the only symptoms which are observed. This makes it difficult to recognize the partial deficiency since the nonspecific symptoms may be brought about by a number of causes, including disease. A good background in the nutrition of poultry, therefore, is necessary for all persons interested in poultry feeding, management, sanitation, and disease.

The nutritive substances of importance in the nutrition of poultry are (1) proteins and amino acids, (2) carbohydrates, (3) fats, (4) minerals, (5) vitamins, known and unknown, and (6) water. The discussions in this chapter include all of the groups of nutritive substances except the vitamins, which are discussed in Chapter 7.

THE PROTEINS AND AMINO ACIDS

The proteins are needed by poultry for the synthesis of new body tissue required in growth, to replace body proteins broken down in maintenance, and to furnish the proteins required for egg formation.

Composition of proteins. Proteins are complex substances composed of amino acids linked together in chemical combination. The amino acids consist of carbon, hydrogen, oxygen, nitrogen, and in two instances, sulfur. Approximately twenty different amino acids have been isolated from proteins. The number of amino acids in each protein as well as the percentage of each is characteristic. Because of this a great many proteins are possible, and

many exist in nature. Every feedstuff contains a number of different proteins.

Digestion of proteins. Proteins are not absorbed from the intestinal tract as such but are broken down during digestion into their constituent amino acids by proteolytic enzymes secreted into the gastric, pancreatic, and intestinal fluids. Some dipeptides, however, are absorbed and split into their constituent amino acids in the intestinal mucosal cells. After absorption the amino acids are then rebuilt into characteristic body and egg proteins.

Protein requirements of poultry. The value of proteins for growth and egg production depends upon their amino acid compositions. Originally it was impossible to use this knowledge because reasonably complete information concerning the amino acid content of the proteins of feedstuffs and the quantitative amino acid requirements of poultry was lacking. Therefore, in investigating the requirements of poultry for protein, extensive studies were made of the supplementary relationships existing between feed proteins. These studies showed that for best results combinations of the mixed proteins of a number of different ingredients were necessary and that, with the exception of soybean protein, the inclusion of some protein of animal origin in the over-all combination of proteins was required. Such a combination of proteins was said to be of good quality, since in all probability the combined proteins of the feed mixtures contained all of the essential amino acids in the right proportions and amounts for maximum growth or egg production.

Experiments on the protein requirement for growth of chicks reviewed by Heuser (1941) and Hill (1944) showed that, when the ration contains a protein mixture of good quality, approximately 20 per cent of protein is required in order to promote rapid growth during the first few weeks of life. The findings presented in these reviews were confirmed in studies reported by Singsen (1947) and Hill (1949). The results showed that as good growth was obtained in crossbred chicks from diets containing either 20 or 21 per cent protein as was obtained from diets containing larger amounts.

At about this time, Heuser et al. (1945) showed that the substitution of feedstuffs of high digestibility for those of low digestibility increased chick growth and egg production, and Scott et al. (1947) reported that combinations of corn and wheat resulted in better growth of broilers, whereas replacing the corn progressively with increasing quantities of pulverized oats depressed growth and the efficiency of feed utilization. The results of these experiments led to the realization that more attention must be given to the energy content of poultry rations than had been done previously.

As a consequence, the quantitative relationship between the available energy in poultry rations and the protein content has been studied extensively by Combs and Romoser (1955), Sunde (1956), Donaldson et al. (1956), Ferguson et al. (1957), Miller et al. (1957), Atkinson et al. (1957), Day and Hill (1957), and Hill and Renner (1957). The results of their experiments showed that the protein requirements of poultry increase as the energy content of the diet increases and that the requirements, therefore, can no longer be expressed as percentages of the diet, but rather should be expressed as energy-protein ratios. The results also showed that the requirement of the chick for 20 per cent of protein during early life applies only to diets of medium energy content. The estimated protein requirements of poultry presented as energy-protein ratios based on the work of these investigators, general knowledge of protein requirements, and relationship of requirements to growth rate, mature size, and rate of egg production are given in Table 6.1.

Other factors which influence protein requirements and thus energy-protein ratios are environmental temperature, the extent to which exercise is restricted, and the finish desired in market poultry. In a

TABLE 6.1

PROTEIN REQUIREMENTS OF CHICKENS AND TURKEYS EXPRESSED AS ENERGY-PROTEIN RATIOS

	Protein Requirement at Medium and High Energy Levels		Approximate Energy-Protein Ratio	
	830–900 PE* 1,240–1,340 ME†	950–1,020 PE 1,420–1,520 ME	Productive	Metabolizable
Chicks				
0–4 weeks ..	20	23	42–43‡	63–65‡
4–8 weeks..	18	21	47–48	70–72
8–12 weeks..	16	19	53–54	78–80
12–20 weeks...	15	17	57–58	84–86
Hens (high production).				
Under 5 lb..	15	17	57–58	84–86
5 lb. and over	14	16	61–62	90–92
Poults				
0–4 weeks..	28	32	30–31	45–47
4–8 weeks..	24	27	36–37	53–55
8–12 weeks	20	23	42–43	63–65
12–16 weeks ..	18	20	48–49	71–73
16–24 weeks..	16	18	53–54	79–81
24 + weeks	13	15	65–66	97–99
Turkey breeders	15	17	57–58	84–86

* Kilocalories productive energy per pound.
† Kilocalories metabolizable energy per pound.
‡ Kilocalories energy per pound divided by percentage protein in the diet

warm environment, less heat is required to maintain body temperature than in a cold environment. This makes possible a somewhat narrower energy-protein ratio. When exercise is severely restricted, as when hens are confined to individual cages, the energy requirement is reduced, and a somewhat narrower energy-protein ratio is desirable in order to prevent the hens from becoming too fat. If the energy-protein ratio is too wide, too much fat may be deposited in the tissues in poultry fed for market, the carcass quality is reduced, and feed conversion is decreased. Thus the estimated energy-protein ratios in Table 6.1 not only may change with the future development of more precise information on the needs of poultry for protein, but also may change with the factors known to affect the protein requirement.

The energy-protein ratios given in Table 6.1 are presented here both as metabolizable energy-protein ratios and as productive energy-protein ratios. Metabolizable

energy (ME) is the energy remaining after subtracting from the gross food energy, as determined by combustion in a bomb calorimeter, the energy losses similarly determined from the undigested residues of food and substances secreted into the gut, and the incompletely oxidized products of the urine. Productive energy (PE) is that portion of the metabolizable energy which is available for the formation of protein and fat in the growing chicken and in eggs. It represents approximately 70 per cent of the metabolizable energy. The remainder is lost largely through the production of heat arising from the chemical reactions occurring in metabolism as a consequence of normal body activities and the maintenance of body temperature.

Fraps (1946) presented the results of an exhaustive study of the productive energy content of poultry feedstuffs, and Hill et al. (1960), Hill and Renner (1960), Potter and Matterson (1960), Sibbold and Slinger (1962), and Stutz and Matterson

(1962) reported values for the metaboliz-able energy content of many of these materials. Hill and Anderson (1958) and Potter et al. (1960) showed that the metabolizable energy values of feedstuffs can be determined more precisely, and thus are more accurate than the productive energy values determined by Fraps (1946).

The variation in the protein requirement of the chick with age is due to the fact that in growth much of the protein in the diet is required for the formation of new body tissue and little is required for maintenance. Because of faster rate of growth the formation of new body tissue by chicks is greatest during the first few weeks after hatching. Thereafter it gradually declines and becomes zero as the chick attains maturity. More protein is therefore required during early chick life than during the period preceding cessation of growth.

The diet of the chick, therefore, does not need to contain 20 per cent protein longer than the first 4 weeks after hatching. Thereafter, the protein content of the diet, according to Heuser (1941), can be reduced by 2 per cent at 4-week intervals but should not be decreased below 15 per cent for pullets during the final period of growth. At this time, when the growth rate is slow, egg production usually begins, which increases the need for protein. The protein requirement of nonlaying chickens, however, probably declines to 12 to 14 per cent before growth finally ceases.

Since the heavier breeds of chickens grow somewhat faster than White Leghorns, their protein requirements should be somewhat greater, particularly during the earlier stages of growth. The results of an investigation carried out in Australia (Anonymous, 1935) showed that chicks of Light Sussex and Australorp breeds made better gains when a high protein ration was fed to 9 weeks of age, while White Leghorns grew equally well when the protein level was reduced at 6 weeks. Mitchell et al. (1926a, 1931), as a result of studies of growth changes in chickens, concluded

that the protein requirement of White Plymouth Rock chicks is greater than that of White Leghorn chicks.

The rations of laying and breeding hens at medium energy levels should contain approximately 15 per cent protein. Heuser (1936) found that a ration containing 12 per cent protein failed to maintain either satisfactory egg production, egg size or body weight, while a ration containing 14 per cent gave satisfactory egg production but did not maintain body weight at all times and was not conducive to best egg size. Satisfactory maintenance of weight and satisfactory egg size, however, were obtained when the ration contained 15 to 16 per cent protein. Results similar to those of Heuser were obtained by Heiman et al. (1936).

The protein requirement varies somewhat, however, with different strains of hens. Some hens have been reported to give satisfactory egg production on rations containing as little as 15 per cent protein. The protein requirement of hens obviously also varies with the rate of egg production. When hens are not laying, the protein requirement is probably in the neighborhood of 12 per cent. The protein requirement of the molting hen does not appear to have been studied. However, it is presumably somewhat greater than that of the nonlaying hen, since the production of a new coat of feathers increases the need for protein. Reviews of the experimental work on the protein requirements of hens have been made by Heuser (1941) and Hill (1941).

The results of research work on the protein requirement of turkey poults, conducted by Funk and Margolf (1932), Headley and Knight (1938), and Mussell et al. (1941), and of experiments conducted at the Cornell University Agricultural Experiment Station reported by Smith and Weaver (1936) indicated that for rapid growth a ration containing at least 24 per cent protein of good quality is necessary. Shortly afterwards, however, Fritz et al. (1947) and Scott et al. (1948).

using rations that were more adequate nutritionally, found that the protein requirement for turkey poults for maximum growth is approximately 28 per cent of the ration. The rations fed the turkey poults in these investigations were of medium energy content.

The protein requirement of poults declines with decreased rate of growth in a manner similar to that of chicks. At medium energy levels the protein content of the diet may be lowered to 24 per cent at 4 weeks of age, 20 per cent at 8 weeks of age, 18 per cent at 12 weeks of age, and 16 per cent at 16 weeks of age until maturity is reached.

In an investigation of the protein requirement of turkey breeders, Jensen and McGinnis (1961) concluded from the results of three experiments that a ration containing 15 per cent protein would be adequate under most conditions. In the third experiment, however, results believed to be satisfactory were obtained with 10 per cent protein, although slightly better egg weight and hatchability of fertile eggs occurred at 12 per cent protein and maximum egg production was attained at 14 per cent protein.

The protein requirement of ducks has not been completely worked out. Horton (1932) observed that ducklings grew at a much faster rate when fed a ration containing 19 per cent protein than when fed one containing 12 per cent. Hamlyn et al. (1934) obtained somewhat better growth in ducklings at 4 weeks of age when rations were fed containing approximately 20 per cent rather than 17.5 per cent protein.

More recently Scott and Heuser (1951) reported better early growth in ducklings supplied rations containing 17 to 19 per cent instead of 15 per cent protein. The difference in favor of the higher protein rations, however, disappeared by the time the ducklings reached 8 weeks of age. The results of additional experimental work conducted by Scott et al. (1959) indicate that the ducklings fed the lower protein ration to market age were of higher fat and lower protein content than the ducklings receiving the higher protein rations.

The protein requirement of starting and growing ducklings, given in the National Academy of Sciences-National Research Council (NAS-NRC) "Nutrient Requirements for Poultry" (1960b), is 17 per cent.

TABLE 6.2

QUANTITATIVE NUTRIENT REQUIREMENTS OF CHICKENS FOR PROTEIN, ENERGY, AND MINERALS*

	Starting Chickens 0-8 wks	Growing Chickens 8-18 wks	Laying Hens	Breeding Hens
Total protein, per cent	20	16	15	15
Metabolizable energy, kcal/lb.	1,280	1,280	1,280	1,280
Productive energy, kcal/lb.	860	860	860	860
Calcium, per cent	1 0	1 0	2.75	2.75
Phosphorus, per cent††	0 6	0 6	0.6	0.6
Sodium, per cent	0 15	0.15	0.15	0.15
Potassium, per cent	0 2	0 16	?	?
Magnesium, mg/lb.	220 0	?	?	?
Manganese, mg/lb.	25 0	?	?	15
Iodine, mg/lb.		0 2	0 2	0 5
Copper, mg/lb.	9 0	?	?	?
Copper, mg/lb.	0 9	?	?	?
Zinc, mg/lb.	20 0	?	?	?

* Obtained in part from NAS-NRC "Nutrient Requirements for Poultry," 1960b.
† At least 0.45 per cent of the total feed of starting chickens should be inorganic phosphorus. Approximately 30 per cent of the phosphorus of plant sources may be considered a part of the inorganic phosphorus.

TABLE 6.3

QUANTITATIVE NUTRIENT REQUIREMENTS OF TURKEYS FOR PROTEIN, ENERGY, AND MINERALS *

	Starting Poults 0–8 wks.	Growing Turkeys		Breeding Turkeys
		8–16 wks.	16–24 wks.	
Total protein, per cent	28	20	16	15
Metabolizable energy, kcal/lb.	1,280	1,280	1,280	1,280
Productive energy, kcal/lb	860	860	860	860
Calcium, per cent	2.0	1 25	1.25	2.25
Phosphorus, per cent †	1 0	0.75	0 75	0.75
Sodium, per cent	0.15	0.15	0.15	0.15
Manganese, mg/lb.	25.0	?	?	15.0
Zinc, mg/lb	25.0	?	?	?

* Obtained in part from NAS-NRC "Nutrient Requirements for Poultry," 1960b.   Approximately 30
† At least 0.5 per cent of the total feed of starting poults should be inorganic phosphorus.
per cent of the phosphorus of plant sources may be considered a part of the inorganic phosphorus.

Ducklings given this quantity of protein may not grow as fast during the first week or two as those receiving 18 to 20 per cent. However, they later will recover from the slight growth retardation and weigh at market age as much as those provided more protein at the start. For economic reasons the general practice at present is to feed ducklings rations containing 18 per cent protein for the first two weeks and 15 per cent from this time to market age. These rations are usually of medium to high energy content.

The quantities of protein required in rations of medium energy content for chickens and turkeys are given in Tables 6.2 and 6.3. The values were taken from the most part from the NAS-NRC "Nutrient Requirements for Poultry" (1960b).

Amino acid requirements of poultry. Some of the amino acids found in proteins are essential for maintenance and growth of the chick and therefore must be present in the ration in adequate quantities. These are listed in Table 6.4. Alanine, aspartic acid, hydroxyproline, norleucine, proline, and serine are synthesized in the body of the chick and may be dispensed with. Cystine and tyrosine are not required ex-

cept for their sparing effect on methionine and phenylalanine respectively. Glycine and perhaps glutamic acid are synthesized to a limited extent but not in sufficient amounts to promote rapid growth.

Although the dispensable amino acids are not nutritionally essential in the ordinary sense, they are needed by the chick in building body protein. A ration which supplies all the essential amino acids in just the right proportions to meet the specific needs for them is not adequate because it does not contain enough amino acid nitrogen to permit the synthesis of all of the protein required for maintenance, rapid growth and egg production.

Almquist (1947, 1952) discussed the available evidence on the quantitative amino acid requirements of chickens and turkeys. Edwards et al. (1956) found that the quantitative requirement of the chick for lysine was 1.1 per cent of a diet containing 20.0 per cent protein. Using pure amino acid mixtures, Klain et al. (1960) and Greene et al. (1960) restudied the quantitative amino acid requirements of the chick. Similar studies with laying hens, using pure amino acids, have been conducted by Johnson and Fisher (1958). The amino acid re-

TABLE 6.4

Essential Amino Acid Requirements of Chickens and Turkeys[a]

Amino Acid	Starting Chicks	Starting Poults	Laying Hens
	(%)	(%)	(%)
Arginine .	1.2	1.6	
Lysine .	1.0	1.5	0.5
Histidine .	0.3	?	?
Methionine .	0.8	0.87	0.53
or			
{Methionine .	0.45	0.52	0.28
{Cystine.. .	0.35	0.35	0.25
Tryptophan .	0.2	0.26	0.15
Glycine .	1.0	1.0	?
Phenylalanine .	1.4	?	?
or			
{Phenylalanine .	0.7	?	?
{Tyrosine .	0.7	?	?
Leucine .	1.4	?	1.2
Isoleucine .	0.6	0.84	?
Threonine .	0.6	?	0.4
Valine .	0.8	?	?
For protein level	20.0	28.0	15.0

[a] The data on the amino acid requirements of chickens and turkey poults were obtained from the NAS-NRC "Nutrient Requirements for Poultry," 1960b.

quirements of laying hens are qualitatively similar to those of chicks, but the quantities of the amino acids needed to meet their requirements are somewhat less than those of rapidly growing chicks, because of the lower total protein requirement.

Information on the amino acid requirements of turkey poults is incomplete. The results of Snetsinger et al (1962), using pure amino acids, and those reviewed by Almquist (1952) indicate, however, that the amino acid requirements of poults are at least qualitatively similar to those of the chick, although the evidence indicates the quantitative requirements may be somewhat greater. This is to be expected in view of the more rapid growth rate and higher protein requirement of poults.

The NAS-NRC (1960b) amino acid requirements of chickens and turkeys are based largely on the reports just discussed. These values are presented in Table 6.4.

The essential amino acid composition of the more common poultry feedstuffs is

presented in Table 6.5. The data given in the table show that the amino acid composition of the mixed proteins of feedstuffs is not uniform. These differences in amino acid composition are of great practical significance since they make it necessary to use several different feedstuffs in building poultry rations in order to meet the amino acid requirements without undue wastage of protein. Attempts to meet the requirement for an essential amino acid by increasing the amount of protein in the ration do not appear to be satisfactory. Grau and Kamei (1950) have shown that as the protein level in the chick ration is increased, the lysine and methionine plus cystine requirements are also increased, but at a slower rate. This indicates that protein in considerable excess is needed in order to meet a slight deficiency of these amino acids.

The data on amino acid content of feedstuffs given in Table 6.5 make it no longer necessary to depend entirely upon the knowledge of the supplementary relationships of protein to obtain protein of good quality in poultry rations. The amino acid content of the ration can now be calculated from the data and compared with the requirement. By doing this, greater assurance is obtained that the mixed proteins in the ration are combined in such a way as to give a protein combination of optimum quality.

Other functions of amino acids. Amino acids fulfil many other functions aside from their main one as building stones for the protein required in growth, maintenance, and egg production. Creatine, which is physiologically essential for the functioning of muscular tissue, is synthesized in the body from arginine, methionine, and glycine. The amino acid, tyrosine, is used in the formation of the hormone, thyroxine, and certain pigments in the feathers of colored fowl. Lysine has been shown to be needed in some indirect way for feather pigment formation in turkeys. Fritz et al. (1946) observed feather achroma in Bronze turkeys fed a corn gluten meal diet which was corrected by the addition of

TABLE 6.5

ESSENTIAL AMINO ACID COMPOSITION OF COMMON POULTRY FEEDSTUFFS*

Feedstuff	Arginine	Cystine	Glycine	Histidine	Iso-leucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Trypto-phan	Tyrosine	Value
Alfalfa leaf meal, 20%	1 0	0.44	1 0	0.42	1 0	1.4	1.0	0.32	1.02	0.92	0.42	....	1.18
Alfalfa meal, 17%	0 6	0.40	0 9	0.38	0 9	1.0	0.9	0.29	0.92	0.83	0.38	....	1.06
Barley, earl, Pacific Coast.	0 6	0.20	....	0.22	0.5	0.8	0.3	0.17	0.64	0.42	0.14	....	0.62
Barley, Pacific Coast.	0 5	0.20	....	0 2	0.39	0.6	0.3	0.13	0.48	0.31	0.11	....	0.46
Bone meal, steamed...	1 9	0.15	....	0 2	....	....	1 3	....	....	0 5	....	....	....
Buttermilk, dried	1	0 16	0 1	0 7	2.7	3 1	2.4	0.7	1 5	1 6	0.5	0.3	2.8
Corn, whole	0 4	0 0	0 3	0.21	2.35	4 4	0 2	0.18	0.45	0 38	0.09	0.3	0.47
Corn distillers dr. solubles	1 4	0 6	1 0	0 8	1 2	2 7	0 5	0 5	1 2	1 0	0 2	1.0	2.2
Corn gluten meal	1 3	0 1	1 4	0 9	2 5	7 2	1 3	1 0	2 9	1 1	0 1	1.0	1 8
Cottonseed meal ...													
Fish meal, menhaden	4 7	0 8	4 5	1 6	4 1	5 0	5 7	1 8	2 7	2 9	0 6	1 6	3 6
Fish meal, sardine	2 7	1 7	5 5	1 4	1 64	4 0	5 7	2 0	1 4	2 1	0 8	0 5	4 1
Fish solubles, condensed	3 4	0 9	6 0	2 2	1 25	3 5	3 8	1 0	1 3	2 4	0 1	0 7	2 6
Hominy feed, yellow	0 5	0 5	0 3	1 0	2 3	5 8	2 8	0 5	2 9	1 6	0 6		4 2
Liver and glandular meal	4 1	0 9	5 6	1 5	3 0	3 7	4 8	1 3	2 9	1 0	0 6		
Meat and bone scrap, 50%	4 2	0 6	6 6	0 9	1 7	3 5	3 4	0 7	1 8	1 8	0 6		2 6
Meat scrap, 55%	5 4	0 6	6 2	0 8	2 0	3 5	3 8	0 8	1 9	1 8	0 3	0 9	2 6
Milk, dried skim	1 4	0 5	2 5	0 9	2 7	3 9	2 8	0 8	1 9	1 4	0 4		2 2
Oats, excluding Pacific Coast	0 8	0 22	0 3	0 25	0 3	0 9	0 4	0 18	0 59	0 44	0 16		0 68
Oat, Pacific Coast	1 0	0 17	0 4	0 19	0 55	0 7	0 4	0 13	0 44	0 33	0 12	0 3	0 51
Oatmeal, feeding													
Sorghum, milo	0 4	0 75	2 6	0 8	0 54	1 4	0 3	0 6	2 1	0 5	0 2	0 7	0 7
Soybean meal, 50%	3 4	0 85	2 9	0 19	1 46	3 1	2 9	0 16	2 47	3 6	0 12		0 53
Soybean meal, 44%	3 2	0 6	2 6	1 1	2 6	4 5	3 0	0 6	2 3	1 7	0 4	1 4	0 41
Wheat, hard red, winter	4 6	0 22		0 26	0 55	1 8	0 4	0 17	0 65	2 36	0 16	0 5	0 53
Wheat, soft, Pacific Coast.	4 0	0 17	0 9	0 2	0 4	0 9	0 4	0 13	0 42	0 28	0 12		0 41
Wheat bran	1 0	0 0	1 0	0 3	0 8	0 9	0 7	0 2	0 5	0 4		0 4	0 7
Wheat standard middlings	1 0	0 2	0 8	0 4	0 7	0 9	0 5	0 2	0 5	0 4		0 2	0 8
Wheat, red dog.	0 4	0 2	0 7	0 2	0 7	1 4	0 4	0 2	0 4	0 8		0 3	0 8
Whey, dried	0 2	0 3	1 7	0 1	1 2	1 3	1 0	0 2	0 4	0 5		0 5	0 7
Yeast, dried brewers...	2 2	0 5		0 2	1 0	3 0	2 1	0 7	0 4	2 1	1 5		2 3

lysine. The picture in Figure 6.1 shows the abnormal white wing feathers of Bronze poults caused by lysine deficiency.

Methionine is a source of methyl groups for methylation processes in the body and either exerts a sparing effect on choline or takes part in the synthesis of choline when other necessary precursors are present in the ration. The amino acid, tryptophan, is converted into nicotinic acid in the developing chicken embryo, according to evidence obtained by Schweigert et al. (1948). Briggs et al. (1946) reported that either tryptophan or nicotinic acid overcomes the growth depressing effects obtained on feeding chicks a low-tryptophan, low-nicotinic acid diet

Effect of heat upon protein quality. Experimental results have shown that moderate heat treatment greatly improves the quality of soybean proteins for poultry by destroying an inhibitor which interferes with digestion. The poor quality of raw soybean protein was found to be due not to the presence of a heat-labile trypsin inhibitor in the bean, but rather to the presence of some other factor which inhibits the rate of growth (Evans et al., 1947; Borchers et al., 1948; Borchers and Ackerson, 1950). The results of Hayward and Hafner (1941), Almquist et al. (1942), and Melnick et al. (1946) indicate that the growth inhibitor slows the rate of digestion of the amino acid methionine relative to the other amino acids. This could account for the retarded growth obtained by feeding raw soybeans, as the absorbed amino acids would not be in proper proportion for efficient protein synthesis.

Prolonged heat treatment at high temperatures, on the other hand, decreases the quality of proteins, including those of soybeans. Experiments indicate that the amino acid lysine is affected most by drastic heat treatment but that arginine, tryptophan, and histidine are also affected (Riesen et al., 1947; Patton et al., 1948a, 1948b). The loss of availability of these amino acids is largely due to combination with reducing sugars, but Evans and Butts (1948) showed that, while a portion of
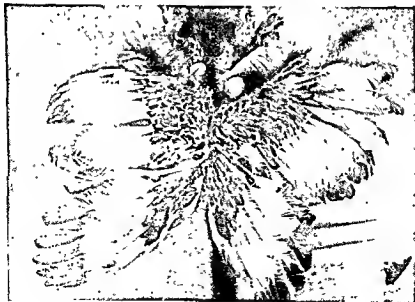
FIG. 6.1 — Lack of normal feather pigmentation in a Bronze poult caused by a deficiency of lysine.

these amino acids is completely inactivated, the rate of digestion of the remainder is slowed up so as to make them incompletely available during a limited digestion period.

Sources of protein. The feedstuffs which are commonly used as protein supplements in poultry rations at the present time are soybean meal, meat scrap, and fish meal. Peanut meal and corn gluten meal are used to a limited extent but are not important sources of protein for feeding poultry. With the development of a method for detoxifying cottonseed meal (Boatner and Hall, 1946; Groschke et al., 1947), it is possible to make this product satisfactory for use as a source of protein. However, none of the vegetable protein supplements except soybean meal contain protein of approximately the same composition of essential amino acids as the proteins of meat scrap and fish meal. The chief amino acid deficiency is lysine. Hence the vegetable proteins are not particularly adapted in general for poultry feeding. Evidence indicates (Heuser et al., 1946), however, that satisfactory growth can be obtained by combining corn gluten meal, cottonseed meal, and peanut meal in limited quantities with soybean meal and with meat scrap and fish meal.

Dried skim milk and dried buttermilk are also excellent sources of protein of superior quality. Their protein content, however, is lower than that of the protein supplements just discussed. They are used in poultry rations more for their content of essential vitamins than for proteins because of relatively high cost.

Influence of protein level upon start of egg production. The protein level of the ration fed to chicks and growing pullets appears to have little effect on the age at which the pullets begin to lay or upon subsequent egg production except when it is low enough to retard growth greatly. Carver et al. (1932), however, reported that a protein level of 12 per cent retarded the age of sexual maturity of White Leghorn pullets from 25 to 40 days, but subsequent results of other workers on the

riboflavin requirements showed that the low-protein ration was greatly deficient in this vitamin. Later Carver et al. (1939) reported that pullets fed rations containing 19 per cent protein reached sexual maturity a few days earlier than those fed rations containing 13 per cent. Several other groups of investigators (Winter et al., 1932; Morris et al., 1932; Heuser and Norris, 1933; Byerly et al., 1933; Tepper et al., 1939) have obtained results indicating that the rate of attaining sexual maturity is not influenced to any marked degree by the protein content of the ration.

Carver et al. (1939) fed protein levels varying from 13 to 19 per cent during the first 22 weeks after hatching and 15.3 per cent thereafter, and found during the first 224 days of production that neither the egg weight nor the rate of egg production was influenced by the quantity of protein fed during the growing period. Bronkhorst (1938) also found that egg yield was not affected by the amount of protein fed growing pullets during the prelaying period.

Protein level and feathering. Because feathers are composed chiefly of protein, it is obvious that poor feathering will result from lack of adequate protein in the ration. Tomhave (1939) showed that when the protein level in the ration was less than 18 per cent during the first 8 weeks, bare breasts occurred in White Leghorn pullets. Several other investigators (Gericke and Platt, 1932; McConachie et al., 1935; Ackerson et al., 1939) reported that feather development improved as the protein level in the ration was increased. A possible relationship between the protein content of the ration and feather pulling, tail picking, and cannibalism was reported by Margolf (1929). In his experiment, these vices developed in chicks fed low protein rations as early as the second and third weeks.

Excess protein. The question as to whether the feeding of excess protein to chickens is harmful remains unsettled. Milne (1932) and McConachie et al. (1935) reported detrimental effects upon growth rate of chicks when the protein

level of the ration was raised to 30 per cent or more. The latter observed high mortality at a 35 per cent level. Lloyd et al. (1949) found that a protein level of 36 per cent in one experiment with turkeys was apparently toxic but that approximately 35 per cent protein gave good results when the ration was a high-energy, low-fiber type. Almquist and Asmundson (1944) obtained improved growth of chicks fed a 30 per cent protein ration.

Uremic poisoning. Patterson (1928) suggested that nutritional gout or uremic poisoning in chickens, except that caused by vitamin A deficiency, may be due to the feeding of excess nitrogenous concentrates. This condition, which is characterized by internal deposits of sodium urate, particularly in the kidneys and ureters, has been observed also by other workers (Mayall, 1929; Hartwigk, 1931; Bird et al., 1946). Each of these workers appears to have a different explanation for the cause of this condition.

In support of Patterson's (1928) suggestion, Schlotthauer and Bollman (1934) reported that they were able to produce gout in turkeys by increasing the protein level of the diet to 40 per cent by the addition of horse meat. They were able to produce this effect also by the addition of 5 per cent urea to the diet. On the other hand, Patton (1939) reported that hens are able to tolerate large single doses of urea.

The injection of large amounts of glycine and of dl-alanine was found by Patton (1939) to be toxic when given to White Leghorn hens. Upon necropsy, the hens fed excess glycine revealed greatly enlarged kidneys which, upon histological examination, revealed indications of incipient necrosis. According to Patton, the evidence demonstrated conclusively that the kidneys are the chief site of glycine toxicity. Patton apparently did not observe an accumulation of urates in the tubules of the kidneys in this work.

Bird et al. (1946) found that a large percentage of the newly hatched chicks from hens receiving a diet deficient in animal protein consistently showed urate deposits in the kidneys and ureters. This condition was prevented by including sardine meal or cow manure in the breeding diet fed the hens. The preventive effects of these products was probably due to vitamin B_{12} content.

CARBOHYDRATES

Carbohydrates, along with fats, provide the energy needed by poultry for growth, maintenance, and reproduction. Carbohydrates, however, play a much more important role than fats in providing energy because they constitute a much larger proportion of the ration.

The nature of carbohydrates, Sugars, starches, dextrins, pentosans, and celluloses are the chief members of a group of organic compounds which are referred to as carbohydrates. All carbohydrates are composed of carbon, hydrogen, and oxygen, the latter two elements always being present in the ratio of two atoms of hydrogen to one atom of oxygen.

The sugars are the structural units from which all carbohydrates are formed. The simple 6-carbon sugars are known as monosaccharides; the more complex sugars, such as sucrose, maltose, and lactose, are known as disaccharides, while the starches and celluloses are composed of many glucose molecules and are therefore termed polysaccharides.

Digestion of carbohydrates. Only the simple sugars have sufficiently small molecular structures to gain entrance into the blood stream from the intestinal system. Therefore, all carbohydrates must be broken down into their simplest constituents before they are used in animal metabolism. The animal tissues secrete enzymes called amylases which split starches and dextrins in the lumen of the intestine into the disaccharide, maltose. Another enzyme, maltase, then breaks down maltose after absorption into the mucosal cells of the intestine into glucose, in which form the carbohydrate passes into the blood stream. Other specific enzymes in the digestive system act upon the other disaccharides,

breaking them down into their structural units, the simple sugars. Following passage into the portal vein, the simple sugars are carried to the liver where they are converted into glycogen. Then, as the demand arises in the body for energy, glycogen is broken down, releasing glucose to be carried by the blood to the site showing the demand. Thus glucose performs the major role in carbohydrate metabolism. When the glycogen stores become filled, the excess sugar resulting from carbohydrate digestion is readily converted into fat and is stored in the various fat depots throughout the animal body.

Cellulose and an accompanying compound, lignin, which together make up the cell wall structure of plants, are not acted upon by any enzyme secreted by animal tissues but can be broken down by certain bacteria. Unlike the ruminant which fosters a host of cellulose-splitting microorganisms within its rumen, the chicken is almost totally unable to derive any benefit from cellulose. The enzymes of the digestive tract of the chicken appear, however, to be as efficient as those of any other animal in breaking down starch and dextrins, provided, of course, that these nutrients are not enveloped by a cellulose membrane which protects them from the action of the digestive juices. Such a condition as this appears to exist in certain feedstuffs such as wheat bran, thereby greatly lowering the available energy content of these feeds as compared with other feeds containing equally as much cellulose but having the starch and sugar fractions accessible to digestion.

Chemical determination of carbohydrates. Nearly 100 years ago Henneberg and Stohmann (1860, 1865), of the Weende Experiment Station in Germany, realized that in order to formulate rations which contained adequate energy, they needed a chemical method for determining the indigestible portion of the carbohydrate fraction. The method which they devised is the one still used in most laboratories for determining the crude fiber content of a feed. In brief, the method entails digestion of the feedstuff first in dilute acid, then in dilute alkali, followed by a determination of the percentage of the feed remaining undissolved.

Since pentosans and lignins which cannot be digested by the chicken are slightly soluble in dilute acids and alkalies, this method does not give a true indication of digestibility, but it is useful because, on the whole, digestibility correlates rather well as an inverse function of the crude fiber content of the feed material.

In the chemical analysis of feeds, the carbohydrate portion is divided into two groups: crude fiber representing the indigestible portion and nitrogen-free extract, the supposedly digestible portion. The nitrogen-free extract contains the sugars and starch and small quantities of indigestible pentosans and lignins. It is determined by adding the moisture, protein, fat, ash, and fiber of a feed together and subtracting the sum from 100. In view of the many possibilities for variability in the composition of the nitrogen-free extract, it cannot be relied upon to give absolutely accurate values for the carbohydrate content of feedstuffs.

Cereals and cereal by-products. The cereal grains are universally recognized as our best sources of available energy-producing carbohydrate. The five most widely used cereals, corn, milo, wheat, barley, and oats, appear to rank in the order mentioned as sources of this nutrient. Experience has shown that the first three cereals can be interchanged at will, provided, of course, that the remainder of the diet is adequate in all nutrients other than carbohydrate. Oats and barley contain too much fiber to be used effectively as the sole source of carbohydrate. Removal of the oat hull, as is done in the manufacture of feeding rolled oats, results in a product having an energy content approximately equal to that of corn.

The by-products resulting from the processing of cereal grains are much inferior to the whole grains as sources of carbohydrate. This is due to the presence of portions of the outer coating of the

cereal grains, which are high in fiber and therefore low in digestibility, in practically all of the by-products. Fraps (1946), for example, has found that oat hulls contain no productive energy for the chick.

Milk and milk by-products. Since the most important carbohydrate in feedstuffs is starch, most feedstuffs can be evaluated as a source of carbohydrate by determining the starch content. An exception to this is found in the case of milk and milk by-products where the carbohydrate is present in the form of the disaccharide, lactose. Although the digestive system of the chicken contains an enzyme or enzymes for the splitting of lactose into its constituent monosaccharides, glucose and galactose, this hydrolysis appears to progress at a fairly slow rate.

Experience has shown that while small amounts, up to 10 per cent, of dried milk by-products have a definite beneficial effect upon growth of chicks, raising the level of lactose in the diet too high causes retarded growth. This effect is quite probably the result of two phenomena. First, the slow rate of hydrolysis of lactose reduces the uptake of sugar by the blood stream and thereby reduces the supply of available energy for growth. In addition, the presence of large amounts of unhydrolyzed lactose in the lower intestines and ceca stimulates the growth of acidophilic microorganisms (Hull and Rettger, 1917). The tremendous multiplication of these microorganisms upsets digestion in the lower intestine and produces a severe diarrhea which flushes out many of the nutrients which might otherwise be absorbed from this site.

FATS

Fats, like carbohydrates, are composed of carbon, hydrogen, and oxygen, and are used by the body as a source of energy. Since fats contain more carbon and hydrogen and less oxygen than do carbohydrates, they contain about 2.25 times as much energy per unit weight.

Digestion of fat. After ingestion, fats

are broken down by enzymes present in the intestinal juices into their constituent parts, the fatty acids and glycerol, in order to be absorbed through the intestinal wall. Some monoglycerides, obtained in the course of fat digestion, are absorbed, however, directly into the intestinal mucosal cells. These constituents, following absorption, are recombined into fat which is carried to all parts of the body by way of the lymph and the blood systems. Much of this fat is probably used directly as a source of energy. Any excess, however, is deposited within the cells of the body and in the fat depots underneath the skin, in the abdominal cavity, and around certain of the vital organs. Conclusive evidence that fatty acids may be converted to some extent into carbohydrates and later used to supply energy is still lacking, but glycerol may be converted into glucose and stored temporarily in the liver and muscle tissues as glycogen.

Special role of fat in poultry nutrition. In addition to serving as a source of energy, fat provides the unsaturated fatty acids, linoleic, linolenic, and arachidonic. These were found to be essential nutrients by Burr and Burr (1930), Burr et al. (1932), and Turpeinen (1938), in work with the rat. Reiser (1950) found that the chick is unable to synthesize linoleic and linolenic acids. Hopkins et al. (1960) and Machlin and Gordon (1961) showed that highly purified methyl linoleate stimulates chick growth. No effect was obtained by the former workers from methyl oleate and none from methyl linolenate by the latter. The quantity of linoleic acid required, however, is probably small under most conditions. Menge and Denton (1961) observed that edible coconut oil which contains little linoleic acid stimulated growth almost as well as equal quantities of corn oil or soybean oil in chicks, fed a simplified diet containing 0.5 per cent corn oil and soybean meal as the chief protein source. Russell et al. (1940) reported that a ration containing 0.1 per cent or less of dietary fat did not significantly retard the growth of

chicks up to 14 weeks of age when care was taken to provide the vitamins removed by the extraction procedure. On the other hand, some fat in the diet is highly desirable. Russell et al. (1942) showed that it aids in the absorption of the all-important fat-soluble vitamins.

Polyunsaturated fats such as corn oil, soybean oil, or safflower oil have been reported to increase egg size by Jensen et al. (1958), Hopkins and Nesheim (1962), and Marion and Edwards (1962). Increased hatchability of fertile eggs from the use of these fats has been reported by Machlin and Dudley (1962) and Marion and Edwards (1962). Shutze and Jensen (1963) obtained evidence that these effects are due chiefly to linoleic acid. The results of studies on the influence of polyunsaturated fats on egg production are conflicting.

Nonedible fats, particularly animal fats, are now used extensively in poultry rations largely because of favorable price resulting from the substitution of detergents for soap. The quantity usually added varies from 2.5 to 5 per cent.

Importance of antioxidants. The unsaturated fatty acids of fats, when not protected adequately by natural or synthetic antioxidants, first lose hydrogen, forming fatty acid-free radicals. Then, through uptake of oxygen, they are converted into organic peroxides which eventually break down into ketones and aldehydes, giving rancid fat its characteristic odor. According to Matill (1927) and Smith (1939) the peroxides may destroy the vitamin E and vitamin A activity of the feed, thereby producing a deficiency of these vitamins even though they were present in ample amounts at the time the feed left the manufacturer. More recent evidence by Miller et al. (1955) indicates that the destructive substance formed in unsaturated fatty acids is the free radical formed by abstraction of hydrogen. This in turn abstracts hydrogen from vitamin E, eventually converting it to the inactive quinone form. The destruction of vitamin A probably proceeds in the same manner but, for the most part, only after the vitamin E in the ration is destroyed. Thus, it is of importance, in the formulation of feed, to use ingredients which contain fat that has not started to become rancid. Vitamin E, which is present in many feedstuffs, is frequently added to poultry rations, is an effective antioxidant, but synthetic antioxidants are also used to prevent the initiation of fatty-acid oxidation. The mineral element selenium which can at least partially take the place of vitamin E in metabolism probably also functions for the most part as an antioxidant. Most of the natural carriers of fat contain unknown antioxidants which are sufficient to protect the fat for a considerable period of time, provided they have not been destroyed by heat or lost in some other way during the processing of the material.

ENERGY

Of the various nutrients needed for growth and production, the requirement for energy is by far the largest. Until recently, however, major attention in feeding poultry was given to the requirements for protein, amino acids, minerals, and vitamins, rather than energy needs. This has been due in part to the fact that a deficiency of energy in a poultry ration does not in general produce definite symptoms of deficiency other than lowered growth and reduced efficiency of feed utilization.

Robertson et al. (1948) presented results which indicated that White Leghorn chicks, for satisfactory growth, required a ration containing approximately 800 kcal. of productive energy per pound of feed. Panda and Combs (1950) obtained evidence which indicated that 850 kcal. per pound of ration were sufficient to promote rapid growth in New Hampshire chicks. The high energy ration reported by Scott et al. (1947) contained about 1,000 kcal. per pound of ration. Hill and Dansky (1954) found that maximum growth in Red Rock crossbred chicks, as determined by weight, was obtained on feeding rations containing as little as 625 kcal. per pound when the

low calorie content was obtained by diluting the ration with pulverized oat hulls. The greatest fat deposition in the bodies of the chicks was obtained, however, when the undiluted ration containing 970 kcal. per pound was fed. The carcass fat, dry basis, of the broilers fed this ration was 26.8 per cent, while the fat content of broilers fed a ration containing 858 kcal. of productive energy per pound was 23.2 per cent. Since this does not appear to be low enough to have any material effect on the quality of the carcass, the productive energy requirement of broilers appears to vary from approximately 850 to 1,000 kcal. per pound. These values on converting to metabolizable energy become 1270 kcal. to 1490 kcal. per pound respectively. At lower productive energy levels the fat content of the carcass was depressed, apparently because the chicks were unable to consume sufficient feed. Such rations, however, are satisfactory for the production of pullets for flock replacement since high fat content of the carcass is not a criterion of pullet quality.

The quantity of metabolizable energy required for egg production has been reported by Hill (1958) to be approximately 350 kcal. per hen per day with rate of lay at 70 per cent. Since the feed requirement for each 10 per cent change in egg production has been estimated by Byerly (1911) to be 0.014–0.015 pounds per hen per day, the change in the daily metabolizable energy requirement for each 10 per cent change in production is approximately 20 kcal., when the diet contains approximately 1350 kcal. per pound. Thus a hen laying at the rate of 90 per cent requires about 390 kcal. per day and one laying at the rate of 50 per cent about 310 kcal.

MINERAL ELEMENTS

The essential mineral elements are as important in the maintenance of the life, well-being, and production of poultry as amino acids and vitamins. They enter into the composition of the bones and give the skeleton, the bony framework of the body, the rigidity and strength needed to support the soft tissues. Minerals combine with protein, lipids, and other substances which make up the soft tissues of the body. They take part in the maintenance of osmotic pressure and the acid-base balance and exert specific effects on the ability of muscles and nerves to respond to stimuli. Minerals are also necessary for the activation of many of the enzymes present in the body.

Essential minerals. The mineral elements which have been found essential for the maintenance of animal well-being are calcium, chlorine, magnesium, phosphorus, potassium, and sodium, and the trace elements, cobalt, copper, iodine, iron, manganese, molybdenum, selenium, sulfur, and zinc. Cobalt is an essential element for ruminants, but the work of Davis et al. (1953) indicates that as long as the diet is adequate in vitamin B_{12}, it is unnecessary for poultry. Fluorine in small amounts is a constant constituent of several tissues, particularly bones. Evidence has been obtained that traces of this element may be essential, or at least beneficial, for some species, but no direct evidence to this effect has been obtained with poultry. The analyses of the individual mineral constituents in the body of mature chickens conducted by Halnan (1936) show that the major portion of the calcium, magnesium, and phosphorus of the body is present in the bones. Chlorine, iron, potassium, sodium, and sulfur, on the other hand, are largely present in muscles, other soft tissues, and body fluids.

Minerals in feedstuffs. The feedstuffs of plant origin are low in chlorine and sodium and, with the exception of alfalfa meal, also low in calcium. They contain relatively large amounts of potassium and reasonable quantities of magnesium and phosphorus. Corn and the feedstuffs of animal origin are low in manganese. With this exception and, in the case of milk products, also iron and copper, the feedstuffs of animal origin are reasonably well supplied with minerals. It is necessary, however, to supplement poultry rations

with additional sodium and chlorine in the form of salt, additional calcium, and, in many instances, phosphorus, zinc, and manganese. Because of the use of large amounts of soybean meal in present-day poultry rations, supplementation with additional iodine is usually indicated, unless large quantities of fish by-products are included in the ration, owing to the fact that soybeans have goitrogenic properties (Wilgus et al., 1941a). This is usually supplied by the use of iodized salt.

Calcium and phosphorus. Calcium and phosphorus are discussed together because of their close association in metabolism, particularly in the formation of bone. In the growing chicken, the major portion of the calcium in the ration is used for bone formation, while in the mature fowl the major portion is used for eggshell formation. Calcium is also essential for clotting of the blood, is required along with sodium and potassium for the normal beating of the heart, and is concerned in the maintenance of acid-base equilibrium.

In addition to its role in bone formation, phosphorus exercises important functions in the metabolism of carbohydrates and fats; it enters into the composition of important constituents of all living cells; and salts formed from it play an important part in the maintenance of the acid-base balance. It is apparently also concerned in calcium transport in egg formation.

The utilization of calcium and phosphorus is dependent upon the presence of an adequate amount of vitamin D in the ration. In vitamin D deficiency the amount of calcium and phosphorus deposited in the bones of growing chicks is reduced and the quantity of calcium in eggshells decreased. If the deficiency is greatly prolonged, these elements may even be withdrawn from the bones of growing chickens and mature fowls.

The quantity of calcium and phosphorus required by poultry is dependent to some extent upon the level of vitamin D supplied in the ration. When large amounts of vitamin D are fed, the amount of calcium and phosphorus in the ration may be reduced. On the other hand, a deficiency of vitamin D can be offset to a considerable extent by increasing the quantity of calcium and phosphorus.

The ratio of calcium to phosphorus in poultry rations may be varied over a fairly wide range without serious harm. However, when either element is present in large excess, it interferes with the absorption of the other from the digestive tract. For the growing chick the most desirable ratio appears to lie between 1.5:1 and 2:1. For laying hens the ratio is considerably wider due to their higher requirement for calcium.

All of the common sources of calcium and most of the sources of phosphorus used in feeding poultry appear to be well utilized. The utilization of the phosphorus in feedstuffs of vegetable origin, however, is dependent in part upon the character of the vitamin D included in the ration. The phosphorus in these feedstuffs is largely in the form of phytin. Singsen et al. (1947) showed that pure vitamin D_3 is more effective in making the phosphorus of phytin available than is the vitamin D present in cod liver oil. When phytin was fed to chicks as the only source of phosphorus in a purified diet, Gillis et al. (1949) found that it provided little, if any, available phosphorus, although the vitamin D content of the diet was in excess of the requirement under ordinary conditions. In work with hens, Common (1940) observed that approximately 75 per cent of the phytic acid of the diet was recovered in the droppings. Gillis et al; (1953) concluded that only about 50 per cent of the phosphorus of phytic acid was available to hens, fed either a purified or a practical diet. McGinnis et al. (1944) reported that more than 16 times as much vitamin D is required to promote normal bone development in chicks when the phosphorus is supplied entirely by means of feedstuffs of vegetable origin than when supplied by equivalent amounts in a purified diet by means of inorganic salts.

According to the results of McGinnis et al. (1944) and Singsen et al. (1947), it

seems probable that some of the phosphorus of phytin is available when supplied by means of natural products. Such products contain an enzyme called "phytase" which hydrolyzes phytin and sets the phosphorus free. However, McGinnis (1944) found that the presence of phytase in natural products does not increase the availability of phytin phosphorus, since no better results were obtained with wheat bran, an excellent source of phytase, than with autoclaved wheat bran in which the enzyme was destroyed.

The minimum calcium requirement of growing chicks was reported to be between 0.66 and 0.86 per cent of the ration by Bethke et al. (1929a), about 0.71 to 0.75 per cent by Hart et al. (1930), and approximately 0.66 per cent by Wilgus (1931). The minimum calcium requirement of chicks appears, therefore, to be approximately 0.7 per cent.

The minimum phosphorus requirement of growing chicks was reported by Bethke et al. (1929b) to be between 0.37 and 0.6 per cent, by Hart et al. (1930) about 0.3 to 0.42 per cent, by Wilgus (1931) 0.5 per cent or less, by Supplee (1935) between 0.26 and 0.5 per cent, and by Watkins and Mitchell (1936) less than 0.5 per cent. More recently, Gillis et al. (1949) found that 0.4 per cent of readily available phosphorus promotes normal bone formation and good growth in young chicks. Therefore, the minimum phosphorus requirement of chicks appears to be approximately 0.4 per cent. In order to get satisfactory bone development at this level, however, the phosphorus must be present in the ration in a highly available form.

The calcium and phosphorus requirements of chicks given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b) are 1.0 per cent and 0.6 per cent, respectively (Table 6.2). The requirements, in addition, specify that the ration must contain at least 0.45 per cent phosphorus in the inorganic form so that not all of the phosphorus in the ration is supplied by means of feedstuffs of vegetable origin.

Norris et al. (1934) found that 1.81 per cent of calcium was sufficient to meet the requirements of laying hens as judged by egg production, eggshell strength, eggshell ash, and the levels of calcium and phosphorus in the blood. At this level of calcium, average egg production of 56 gm. eggs in two experiments varying in length from 44 to 48 weeks was approximately 56 per cent, eggshell calcium per egg was 2.09 gm. and eggshell breaking strength 4.09 kg. These values were not increased by raising the calcium content of the diet to 2.13 per cent. Blood calcium was also maximal at 1.81 per cent calcium. In this work, when 3.33 per cent of calcium was included in the ration, a decrease in egg production was obtained and the hens failed to maintain their weight as well as those fed somewhat lower levels of calcium. Gutkowska and Parkhurst (1942) reported that a ration containing 3.9 per cent calcium affected egg production detrimentally, but not hatchability and fertility. The phosphorus content of the ration used in the investigation was 0.75 per cent. Titus et al. (1937), on the other hand, found that levels of calcium varying from 4.05 to 5.4 per cent brought about a decrease in hatchability as well as egg production. Norris et al. (1934) used oystershell flour as the chief source of calcium in their experimental work; Gutkowska and Parkhurst (1942), pulverized calcite; and Titus et al. (1937), ground limestone and gypsum.

Evans et al. (1944b), who studied the calcium and phosphorus requirements of White Leghorn pullets kept in laying cages showed that the best egg production was obtained when the pullets received 2.5 per cent calcium. The duration of this experiment was 16 weeks. In a second experiment of 40 weeks' duration egg production and egg weight at 2.5 per cent calcium were equal to the results obtained with 3.0 per cent and 3.5 per cent calcium and eggshell smoothness tended to be better. Eggshell weight and shell thickness, however, were greater at the higher amounts of calcium. In this experiment egg weight was

somewhat greater than that obtained by Norris *et al.* (1954). This may explain partially the higher calcium requirement reported by Evans *et al.* (1944b). Since oyster shells rather than oystershell flour were used as the source of calcium in their experiments, it is probable also that the oyster shells were used less efficiently because of large particle size.

In the work by Norris *et al.* (1934) the percentage utilization of calcium for eggshell formation was 61.9 per cent when the diet contained 1.81 per cent, the quantity found to be optimum under the experimental conditions. This is in excellent agreement with the percentage retention of approximately 60 per cent observed by Hurwitz and Griminger (1960) in hens fed a diet containing 2.7 per cent calcium. These hens laid eggs, averaging 59.8 gm., at a rate of 77.0 per cent for 44 weeks. Since little calcium is stored by mature, heavily laying hens, percentage calcium retention and percentage utilization for eggshell formation are very nearly identical.

The amount of calcium in the shell of a 58–60 gm. egg is about 2.2 gm. At 60 per cent utilization for eggshell formation, the quantity of calcium which must be ingested per hen per day can be calculated from the rate of lay. This is about 1.83 gm. for 50 per cent production, 2.57 gm. for 70 per cent, and 3.30 gm. for 90 per cent. The calcium content of rations of medium energy content, designed to meet the needs of hens averaging 4.5 pounds at the end of the first production cycle, can be estimated from data reported by Byerly (1941). Such rations should contain 1.8 per cent calcium for an egg production rate of approximately 50 per cent, 2.25 per cent for 70 per cent production, and 2.75 per cent for 90 per cent production. Slightly larger amounts will be required in rations of high-energy content containing added fat, or in rations fed to hens of lower final average weight than 4.5 pounds.

Norris *et al.* (1934), using a ration containing 1.8 per cent calcium, found that the minimum phosphorus requirement of laying hens was 0.75 per cent of the diet. Miller and Bearse (1934), with rations varying in calcium from 2.23 to 3.03 per cent, obtained the highest egg production with 0.8 per cent phosphorus. Evans *et al.* (1944a) obtained higher egg production with 0.8 per cent phosphorus in the ration of laying hens than when 0.6 per cent was supplied. The calculated available phosphorus content of the diets used by the research workers which revealed minimum requirements were 0.45–0.50 per cent, 0.50–0.60 per cent, and 0.45–0.50 per cent respectively. Gillis *et al.* (1953) reported that hens confined on wire-mesh floors for experimental periods up to 35 weeks required 0.5 per cent available phosphorus for maintenance of weight and egg production and 0.6 per cent for optimum blood phosphorus level and prevention of bone decalcification. Singsen *et al.* (1962) showed that hens confined on wire-mesh floors for 40 weeks required more than 0.35 per cent available phosphorus but not more than 0.55 per cent for egg production. Hatchability of fertile eggs and percentage of bone ash, however, were satisfactory at the smaller amount.

An exceptionally high phosphorus turnover is a characteristic of the rapidly laying hen. Not only is the excretion of phosphorus through the usual channels increased but, in addition, appreciable amounts are deposited in the egg. Phosphorus is found in the egg yolk as a constituent of lecithin and vitellin. Phosphorus is also concerned indirectly in eggshell formation although it comprises less than 1 per cent of the shell. Norris *et al.* (1933) found that egg production per hen was not only decreased when the phosphorus content of the diet was 0.5 per cent, but also that the amount of ash in the eggshell was decreased. Halnan (1925) pointed out that egg production is associated with increased phosphorus catabolism, and that during egg production the phosphorus lost from the body is much greater than that contained in the eggs laid. Halnan's observations were confirmed by Common

(1932), who found that egg production was correlated with relatively heavy excretion of phosphorus in the feces.

The calcium requirement of laying hens given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b) is 2.75 per cent of the ration and the phosphorus requirement 0.6 per cent (Table 6.2).

Mussehl and Ackerson (1935) found that to obtain maximum growth and maximum bone ash in turkey poults, levels of calcium varying from 1.45 to 1.98 per cent and levels of phosphorus varying from 0.63 to 1.02 per cent were required in a ration containing 1.0 per cent of cod liver oil. Motzok and Slinger (1948) presented evidence which indicated that turkey poults fed a high-energy ration required minimum levels of 1.3 to 1.6 per cent calcium and 0.7 to 0.9 per cent phosphorus. Hammond et al. (1944) reported that 1.0 per cent calcium and 0.6 per cent phosphorus are adequate for growing turkeys and that under favorable conditions as little as 0.5 per cent phosphorus may be fed without detrimental effect. Singsen et al. (1947) showed that 0.65 per cent nonceral phosphorus promoted satisfactory growth and bone formation in turkey poults and that 0.4 per cent was adequate, provided the diet contained not less than 0.65 per cent total phosphorus. Wilcox et al. (1955) obtained results indicating that the phosphorus requirement of turkey poults fed a practical ration was 1.0 per cent, when approximately 0.45 per cent was phytin phosphorus and the remainder inorganic. No studies appear to have been conducted on the calcium and phosphorus requirements of breeding turkeys.

The calcium requirements of turkey poults given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b) is 2.0 per cent of the ration and the phosphorus requirement is 1.0 per cent (Table 6.3). The calcium and phosphorus requirements given in the report for breeding turkeys are 2.25 and 0.75 per cent of the ration, respectively, in spite of the lack of experimental evidence. These requirements are based, presumably, upon the fact that breeding chicken rations have promoted satisfactory egg production in breeding turkeys. The NAS-NRC requirements also specify that the ration of starting poults contain 0.5 per cent inorganic phosphorus in order to have some highly available phosphorus in it.

Magnesium. Magnesium is closely associated with calcium and phosphorus in the body. It is essential for bone formation, about two-thirds of the magnesium in the body being present in the bone, chiefly as a carbonate. It is also necessary for carbohydrate metabolism and the activation of several enzymes. Eggshells contain about 1.4 per cent magnesium.

Magnesium is present in sufficient amounts in ordinary feedstuffs so that practical poultry rations contain in general enough to meet the requirement. It is possible, however, to formulate rations which contain excess magnesium, with the result that detrimental effects are produced. Buckner et al. (1932) found that the addition of magnesium carbonate to chick rations in amounts sufficient to raise the magnesium level from 0.76 to 7.05 per cent upset the calcium and phosphorus balance, resulting in deformed bones with low ash and calcium content. A similar effect on bone ash has been reported by Mussehl et al. (1930), while Schaible et al. (1963) obtained perosis with magnesium carbonate additions. Alder (1927) found that feeding dolomite limestone containing a high percentage of magnesium for a period of four months caused egg production to decrease and the shells of the eggs to become progressively thinner. Nearly every hen in the dolomite pen developed diarrhea as indicated by the droppings and badly soiled condition of the feathers on the abdomen. The hens also became extremely irritable and easily frightened. All of these conditions cleared up within a short time after substituting a high-grade limestone for the dolomite. Work with several species of animals, however, indicates that magnesium is less

harmful when the ration contains liberal amounts of calcium and phosphorus than when these elements are fed at a marginal level.

Almquist (1942) found that the magnesium requirement of the chick is approximately 0.04 per cent during the first few weeks of life. Almquist observed that the chicks fed the basal ration grew slowly for approximately one week, then ceased growing and became lethargic. When disturbed, these chicks frequently passed into a brief convulsion accompanied by gasping and finally into a comatose state sometimes ending in death. Gardiner et al. (1959) reported that the magnesium requirement of the chick for maximum growth and prevention of deficiency symptoms is approximately 0.025 per cent. Using a purified diet containing 0.7 p.p.m. magnesium, McWard and Scott (1961) found the chick's magnesium requirement for good growth and survival is approximately 0.02 per cent. The best growth, however, was obtained at magnesium levels of 0.025 per cent to 0.045 per cent. Nugara and Edwards (1961) showed that the magnesium requirement of the chick is affected by the dietary levels of calcium and phosphorus. They found that, when the calcium content of the diet was 1.2 per cent and the phosphorus 0.6 per cent and 0.9 per cent, the magnesium requirement was 0.029 per cent and 0.042 per cent respectively. The higher values obtained by these investigators agree reasonably well with the magnesium requirement of the chick given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b). This is 220 mg. per pound or 0.048 per cent.

The magnesium requirement of turkey poults fed a purified diet, was reported by Sullivan (1962) to be 216 mg. per pound or 0.0475 per cent. Keene and Combs (1962) obtained results which indicated that the poult, fed a purified diet containing an antibiotic, required 0.042 per cent magnesium. In work with chicks fed a purified diet, the inclusion of an antibiotic in the diet was found to decrease the chick's requirement for magnesium slightly. The magnesium deficiency symptoms of poults were observed to be similar to those of chicks Poults showed no evidence of toxicity when the diet contained 0.18 per cent magnesium.

Sodium and chlorine (salt). Sodium as chloride, carbonate, and phosphate is found chiefly in the blood and body fluids Sodium chloride is the chief inorganic constituent of the blood plasma, and is presumably the source of chlorine in the hydrochloric acid of the gastric juice Sodium is connected intimately with the regulation of the hydrogen ion concentration of the blood. Sodium, along with potassium and calcium in proper balance, is essential for heart activity.

The addition of salt to poultry mash mixtures is common practice and in most instances is probably necessary for optimum growth and production. Mitchell and Carman (1926) reported that chicks fed a cereal ration containing no added salt showed retarded growth with decreased efficiency of food utilization. They concluded that the retarded growth was due to a deficiency of sodium rather than of chlorine. These results have been confirmed by Prentice (1933a) and by Burns et al. (1953). Prentice (1933b) also reported that a lack of salt in the ration of laying hens resulted in decreased egg production and egg size, loss of weight, and cannibalism.

Sjollema (1935) and Halpin et al. (1934) obtained better growth in chicks when fed rations containing 0.5 and 1.0 per cent added salt. Burns et al. (1952) found that the minimum amount of salt required by hens fed a purified diet was approximately 0.2 per cent but suggested 0.5 per cent as an optimum amount to supply. Burns et al. (1953) reported that the minimum amount of chlorine required by chicks fed a purified ration is less than 0.06 per cent and the amount of sodium is between 0.1 and 0.3 per cent. These values correspond to 0.25–0.75 per cent salt. Leach and Nesheim (1963) observed that

chicks fed a purified diet containing 0.24 per cent sodium and 0.4 per cent potassium required 0.12 per cent chlorine.

Slinger et al. (1950) obtained evidence that maximum growth in chicks fed high-energy, low-fiber rations was obtained with 0.25 per cent or less of added salt but that the salt requirement was increased on including wheat by-products and cellulose in the ration. Heuser (1952) obtained maximum growth in chicks by supplementing a medium-energy ration already containing 0.4 per cent chlorides expressed as salt with 0.25 per cent salt. No further improvement in growth was observed with larger amounts of salt.

According to the NAS-NRC "Nutrient Requirements for Poultry" (1960b), 0.15 per cent of sodium should be adequate for all ages of chickens and turkeys. This is equivalent to 0.37 per cent sodium chloride. In view of the presence of some sodium in practical ingredients, the addition of 0.25 per cent salt is, however, usually sufficient. This represents added salt and not salt already present in the ingredients of the mash mixture. When the ration is composed of both mash and grain, the mash mixture should contain 0.5 per cent added salt in order to provide approximately 0.25 per cent in the entire ration. However, these recommendations may supply too much salt and lead to excess water consumption and wet litter when large amounts of animal products which contain considerable quantities of salt are included in the ration. Under these conditions it seems advisable to consider the salt already present in the ration and add enough to provide a total of approximately 0.37 per cent.

Excessive amounts of salt in the ration are toxic to chickens. Sufran (1909) found that the lethal dose is approximately 4 gm. per kilogram of body weight. Quigley and Waite (1932) fed chicks rations containing from 1.0 to 15.0 per cent salt and found that levels of 8.0 per cent or greater depressed growth. Mortality, however, was excessive above the 5.0

per cent level. These workers confirmed the observation of Suffran that the minimum lethal single dose is 4 gm. per kilogram of body weight. Barlow et al. (1948) fed day-old chicks graded amounts of salt in an all-mash ration to nine weeks of age and observed mortality of 18.8 per cent at the 3.0 per cent salt level and 34.4 per cent at the 4.0 per cent level. Young chicks were more susceptible to toxic effects of salt than older ones and water consumption increased with increase in the salt content of the ration. Mitchell et al. (1926b) found that chickens from 9 to 21 weeks of age could be fed as much as 8.0 per cent salt in the ration without any detrimental effects on their rate of growth and physical condition after the chickens became accustomed to the ration. Matterson et al. (1946) fed day-old turkey poults graded quantities of salt in an essentially salt-free diet for 23 days and observed 25 per cent edema and 25 per cent mortality at 4.0 per cent salt but none at 2.0 per cent. Roberts (1957) found that a ration containing 4.0 per cent salt did not affect either growth or food consumption in 8-week-old poults but water consumption was noticeably increased. In mature turkeys 8.0 per cent salt may have caused some reduction in gain but food consumption was not decreased. No mortality occurred in any of the experiments. Torrey and Graham (1935) reported that ducks are more susceptible to salt poisoning than chickens.

The symptoms of salt intoxication are inability to stand, intense thirst, pronounced muscular weakness, and convulsive movements preceding death. Postmortem examination has revealed lesions in many organs, but particularly hemorrhages and severe congestion in the gastrointestinal tract, muscles, liver, and lungs.

Potassium. Potassium is widely distributed in feedstuffs of both plant and animal origin, and although the requirement of poultry for this element is rather high, there seems but little likelihood of a defi-

ciency occurring in practical poultry rations.

Potassium, in contrast to sodium, is found primarily in the cells of the body rather than in the body fluids. The soft tissues of the fowl contain more than three times as much potassium as sodium. The sodium and potassium content of the bones are approximately the same. The fundamental role of potassium in metabolism is not well understood. It is necessary for normal heart activity where it exerts an effect opposite that of calcium, reducing the contractility of the heart muscle and favoring relaxation. Potassium ions also appear to increase membrane permeability.

Ben Dor (1941) reported that chicks deficient in potassium exhibit high mortality and retarded growth. He concluded that the chick requires 0.17 per cent potassium in the diet. Gillis (1948, 1950) observed that an interrelationship exists between potassium and phosphorus. He found that although 0.16 per cent potassium was sufficient to prevent mortality, 0.2 per cent potassium was required when the phosphorus level was 0.6 per cent, and 0.24 per cent potassium was needed when the phosphorus level was 0.55 per cent. Burns et al. (1953) reported that the potassium requirement of chicks was between 0.25 and 0.40 per cent, the higher level being required for maximum growth. Leach et al. (1959) obtained evidence that the minimum potassium requirement for rapid growth of chicks fed a high energy diet containing 0.68 per cent phosphorus is 0.3 per cent. Results obtained by Supplee and Combs (1960) indicate that the potassium requirement of turkey poults is approximately 0.6 per cent of the diet in the absence of an antibiotic and 0.45 per cent in the presence of one. The potassium requirement of starting chicks given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b) is 0.2 per cent and that for growing chicks is 0.16 per cent (Table 6.2).

Manganese. Manganese is one of the so-called "trace elements" required in poul-

try. Wilgus et al. (1936, 1937a) reported that perosis in poultry is caused for the most part by manganese deficiency. They also showed that the perosis-preventing property of certain feedstuffs is correlated with their manganese content.

Perosis is an anatomical deformity of the leg bones of young chickens, turkeys, pheasants, grouse, and quail. The symptoms generally found are gross enlargement of the tibio-metatarsal joint, twisting or bending of the distal end of the tibia and of the proximal end of the metatarsus, and, finally, slipping of the gastrocnemius tendon from its condyles (Fig. 6.2). The latter symptom causes complete crippling in the affected leg, and if both legs are so affected, death usually results due to the inability of the chick to secure food and water. Early observers of perosis noted that it was most likely to occur under the crowded conditions of confinement rearing; that rations having a high mineral content tended to aggravate the condition; and that heavy breeds were more susceptible than light breeds.

The amount of manganese required by chicks varies with different breeds and strains. Wilgus et al. (1937a) reported that 55 p.p.m. was adequate for the cross-

FIG. 6.2 — An extreme case of perosis. The most common causative factor of this disease is manganese deficiency.

bred chicks used in their experiments. Insko et al. (1938) found 36 to 37 p.p.m. to be adequate for growth and the prevention of perosis in Rhode Island Red chicks. Gallup and Norris (1939a) reported that approximately 50 p.p.m were required by New Hampshire chicks, while 30 p.p.m. completely prevented the disorder in White Leghorn chicks. They also observed that 1,000 p.p.m. of manganese was not toxic to day-old chicks, kept on experiment to six weeks of age.

In addition to its perosis-preventing properties, manganese is necessary for the formation of normal bones. Wilgus et al. (1937b) observed that frequently the leg bones of chicks fed perosis-producing diets were thickened and shortened. Gallup and Norris (1938) reported that the leg bones of chicks fed a manganese-deficient diet were 7 or 8 per cent shorter than those of chicks of the same age, sex, and weight receiving adequate manganese. In a more extensive study, Caskey et al. (1939) reported that manganese deficiency in the diet of chicks resulted in a highly significant shortening of the bones of the legs and wings and also a shortening of the spinal column. They also found that the ash content of the bones of the deficient chicks was significantly lower than that of chicks fed an adequate diet.

The evidence that manganese is necessary for bone formation is supported by the observations of Wiese et al. (1939, 1941) that bone and blood phosphatase, as well as the ester phosphorus of the blood, are depressed in manganese deficiency in the chick. Combs et al. (1942) confirmed the observation that manganese deficiency lowers bone phosphatase. These results, together with those of Caskey et al. (1939), suggest that manganese deficiency causes a disproportionately greater retardation in the development of bones in chicks during growth than that of other tissues of the body. Creek et al. (1960) have shown, however, that the stress of the relatively greater weight of the soft tissues is not the primary cause of the bone malfor-

mation in perosis. After severing the Achilles tendon at the right hock joint at three days of age, these investigators observed that malformation of the bones of the immobilized leg developed but to a lesser extent than in the unoperated right leg of other chicks.

The need for manganese by mature fowls was demonstrated by Gallup and Norris (1937, 1939b), who reported that pullets fed a diet containing 13 p.p.m. manganese produced less than half the number of eggs laid by similar pullets fed a diet containing 200 p.p.m. In this work the manganese content of the eggs from the low-manganese group was reduced, fertility was slightly decreased, and the hatchability of eggs was markedly lowered by manganese deficiency. Embryos in the eggs of low-manganese content usually died during the final stages of incubation.

Lyons and Insko (1937) also found that manganese deficiency resulted in very low hatchability of fertile eggs and, in addition, observed the production of chondrodystrophy in the embryos. The peak of mortality for such embryos occurred on the twentieth and twenty-first days of incubation. The chondrodystrophic embryos were characterized by very short, thickened legs, short wings, "parrot beak," globular contour of head, protruding abdomen, and retarded down and body growth. Very marked edema was noted in about 75 per cent of these embryos. The manganese content of the eggs producing chondrodystrophic embryos was much smaller than that of normal eggs.

Caskey et al. (1944) reported that chicks hatched from eggs produced by hens fed a diet deficient in manganese sometimes exhibit ataxia, particularly when excited. This is a nervous disorder involving spasms in which the head may be drawn forward and bent underneath the body or retracted over the back. The ataxic chicks grew normally and reached maturity but failed to recover completely from the ataxia. Caskey and Norris (1940) observed that they also retained the short

bones characteristic of embryos and newly hatched chicks when the maternal diet is deficient in manganese.

Caskey and Norris (1938) reported that the breaking strength of the eggs from lots of hens receiving different levels of manganese increased as the quantity of manganese in the diet increased. The breaking strength averaged 6.6 pounds for a lot receiving 6.5 p.p.m. manganese and 9.3 pounds for a lot receiving 100 p.p.m. The ash content of the eggshell also increased with increase in the manganese content of the diet. Lyons (1939) described the effect of a low-manganese diet on the characteristics of eggshells. Eggs produced by hens fed a deficient diet showed large areas of poor calcification as indicated by differences in smoothness and translucency. A lowered breaking strength and a smaller percentage of shell were also found.

In studies on the manganese requirement of hens, Caskey and Norris (1938) concluded that 20 p.p.m. is sufficient for egg production and hatchability. The results of Schaible et al. (1938) indicated that additional manganese above 39 p.p.m. did not benefit fertility, hatchability, or egg production. Golding et al. (1940) found that heavy breeds require more manganese than light breeds. The work with hens, therefore, supports the results obtained with chicks. Golding et al. (1940) also found that 9 p.p.m. of dietary manganese were adequate for hatchability and the prevention of chondrodystrophy but not for egg production in White Leghorn pullets. This amount of manganese, however, was not sufficient for hatchability in Barred Plymouth Rocks. Couch et al. (1947) obtained evidence which indicated that the manganese requirement of hens in their second year of production is greater than that of pullets. These workers also reported that high levels of manganese in the diet lower the vitamin D requirement.

According to the work of Schaible et al. (1938), manganous sulfate, manganous chloride, manganous carbonate, potassium permanganate, and manganese dioxide appeared to be equally satisfactory sources of manganese for use in poultry rations. Oxide ores of manganese, such as manganite and pyrolusite, were comparable to the foregoing compounds; but the carbonate ore, rhodochrosite, and the silicate ore, rhodonite, were not satisfactory.

Although high concentrations of manganese are poisonous, the amounts used in practice are much below the toxic level. More than thirty to forty times the amounts required were fed by Gallup and Norris (1939a) without apparent harm.

According to the NAS-NRC "Nutrient Requirements for Poultry" (1960b), chicks and poults should receive 25 mg. per pound of ration and hens and turkey breeders 15 mg. per pound. This is usually supplied by including in the ration 6 to 8 ounces of a feed grade of manganese sulfate containing about 70 per cent of the pure compound.

Iodine. Traces of iodine are required for normal functioning of the thyroid gland in poultry as well as all other animals. Most of the iodine in the body is held by the thyroid which has a remarkable affinity for this element. Thyroxin, the hormone secreted by the thyroid, contains approximately 65 per cent iodine and acts as an important regulating agent in body metabolism. When the intake of iodine is suboptimal, the thyroid tissue enlarges and the condition known as goiter results

The problem of iodine deficiency is confined to areas in which the soil, and consequently the water and feed crops, contain insufficient amounts of this mineral. In the United States these areas are primarily in the Northwest and in the Great Lakes region, but many other sections are either marginal or actually deficient in this respect. To some extent iodine deficiency in poultry has probably been offset by the widespread use of fish meal, oyster shells, and fish oils which contain significant amounts of iodine. Nevertheless, goiter has been described in poultry

by a number of investigators. Welch (1928) reported that goiter occurred frequently in poultry in Montana. He minimized, however, the effect of this condition on the general health and productivity of the affected birds. Wilgus et al. (1941a) observed that soybeans in the diet of poultry have a goitrogenic effect. They found that this effect is partially inactivated by heat, however, and that iodine is a counteractant. Soybean meal was also found to be goitrogenic, but no detrimental effects other than that on the thyroid gland were noted in its use in feeding growing chickens.

Patton et al. (1939), Wilgus et al. (1941b), and Gassner and Wilgus (1940) reported that iodine deficiency results in enlarged thyroids and in some cases lower body weight in growing chicks. They estimated that the growing chicken requires 1 p.p.m. of iodine in the diet for optimum growth and the prevention of goiter. They observed congenital goiter in baby chicks hatched from hens receiving 0.025 p.p.m. of iodine in the ration. By using more reliable procedures for determining iodine, Godfrey et al. (1953) found that the iodine requirement of chicks is between 0.03 p.p.m and 0.15 p.p.m. Later, Creek et al. (1957) reported that 0.05 p.p.m. or less failed to support normal growth while 0.075 p.p m. was sufficient. Larger amounts, however, were required for normal thyroid histology, since abnormalities were prevented only when the level was as high as 0.40 p.p.m. The inclusion of 4.8 p.p.m. of iodine in the diet in preliminary studies was not found to be toxic.

The results of studies on the iodine requirement of breeding hens by Rogler et al. (1961) showed that hatchability of the eggs of hens fed a ration containing 0.010–0.024 p p m. of iodine was greatly reduced and hatching time delayed 2 to 2.5 days. Embryonic development except for thyroid size appeared normal, however, when the iodine level was increased to 0.035 p.p.m. At 0.125 p.p.m. of iodine thyroid size became essentially the same as when the diet of the hens contained 0.5 p.p.m.

The results of these workers indicate that the use of 0.5 per cent iodized salt in chick rations should prevent the development of iodine deficiency, since this would supply 0.35 p.p.m. in addition to that contained in the diet. In the NAS-NRC "Nutrient Requirements for Poultry" (1960b) the iodine requirement of chicks and breeders is estimated to be 0.5 mg. per pound and that of growing chicks and layers to be 0.2 mg. per pound.

Iron and copper. Both iron and copper are necessary for hemoglobin formation. Iron is present in heme, the iron porphyrin nucleus of hemoglobin. This nucleus is also one of the components of the cytochromes and the enzymes, peroxidase and catalase. Copper also takes part in the activity of several enzymes, but, while essential for hemoglobin formation, does not enter into its composition. In the absence of copper, dietary iron is absorbed and deposited in the liver and elsewhere, but hemoglobin formation does not occur, and anemia results. Elvehjem et al. (1929) showed that both iron and copper are required for hemoglobin synthesis in the chick. Hill and Matrone (1961) concluded from the results of several experiments that the chick needed approximately 40 p.p.m. of iron and 4 p.p.m. of copper for maximum hemoglobin formation (Fig. 6.3). Davis et al. (1962) presented evidence which indicated that the iron requirement of the chick was approximately 65 p.p.m. when the diet contained 10 p.p.m. of copper.

The demand for iron and copper for egg formation is large, as the average egg contains about 1.1 mg. of iron and 0.067 mg. copper. The results of several investigators indicate, however, that the addition of extra iron and copper to practical laying rations does not increase the iron and copper content of the eggs, although Erikson et al. (1933) found that hens with access to sunshine and bluegrass
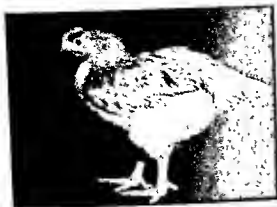
FIG. 6.3 — Deficient pigment formation in the feathers of a New Hampshire chick resulting from a shortage of dietary iron. The chick was also anemic.

range, produced eggs with higher iron and copper content than hens confined indoors.

The hemoglobin level of the blood of hens has been observed to fall with the beginning of egg production, but this does not appear to be related to the iron and copper content of the diet. In view of the fact that the hemoglobin level rises rapidly with the onset of broodiness, it appears more probable that the low hemoglobin levels which prevail in egg production are due to changes in the hormone mechanisms of the body rather than to iron and copper deficiencies.

Mayo et al. (1956) studied the copper tolerance of young chicks using growth, mortality, and occurrence of muscular dystrophy as indications of copper tolerance. In one experiment, 324 p.p.m. of copper caused significant retardation of growth at four weeks of age, and in a second experiment, 520 p.p.m. decreased growth at this age, but the males equalled the weight of the control lot when eight weeks of age. A marked increase in mortality occurred when the diet contained 1,270 p.p.m. of copper. The estimated iron and copper requirements of the chick given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b), are respectively 20 and 2 p.p.m.

Sulfur. The amino acids, methionine and cystine, which contain sulfur in organic combination, supply practically all the sulfur utilized by poultry. Traces, however, are also supplied by the vitamins, thiamin and biotin, and by inorganic sulfates. Work by Machlin (1955) as well as by Gordon and Sizer (1955) indicates that the chick has a requirement for sulfur which may be satisfied either by providing methionine and cystine in excess of that required for protein formation, or by means of sulfates. When the methionine and cystine content of the diet was somewhat deficient, sulfate was found to exert a sparing effect on these amino acids.

With adequate quantities of methionine and cystine in the diet, all the other sulfur-containing compounds in the body appear to be derived from the catabolism of these amino acids. In studies on the sulfur content of egg yolk and egg white, Marlowe and King (1936) found that all of the sulfur in these products was organically bound, and also that nearly all could be accounted for by the cystine and methionine sulfur.

Zinc. Traces of zinc appear to be necessary for life in all animals. It is a constituent of the enzyme carbonic anhydrase and appears necessary for the activation of several enzymes. O'Dell and Savage (1957a, 1957b) reported significant increases in the growth of chicks fed a purified soybean protein diet containing 15 to 20 p.p.m. of zinc by supplementing the diet with 100 p.p.m. of this mineral. Zeigler et al. (1958) observed zinc deficiency in chicks supplied demineralized water and either a purified soybean protein diet or a purified casein diet containing respectively 9 and 4.2 p.p.m. of zinc. The gross deficiency symptoms were retarded growth, poor feathering, enlarged hocks, and scaling of the skin, particularly on the feet. These symptoms were obtained only when the galvanized feeders and waterers were replaced with stainless steel ones, the galvanized battery cages coated with plastic resin, and the diets

stored in tin cans. Without these changes, little evidence of deficiency was obtained. Increasing the calcium content of the diet from 1.23 per cent to 2.07 per cent enhanced the severity of some of the symptoms. Similar observations have been reported by Edwards et al. (1958).

On feeding graded quantities of zinc to chicks Zeigler et al. (1961) found that the requirement was 12–14 p.p.m., when a purified diet containing casein was fed and 27–29 p.p.m., when the diet contained isolated soybean protein. Davis et al. (1962) reported that chicks fed a diet containing isolated soybean protein required 43 p.p.m. of zinc for maximum growth and bone formation. Evidence was obtained by Kratzer et al. (1959) that the turkey poult required approximately 60 p.p.m. of zinc when supplied a diet containing isolated soybean protein. The requirement was greatly reduced by including the chelating agent, ethylenediaminetetraacetic acid, in the diet. This finding was confirmed by Davis et al. (1962), using chicks as the experimental subjects. In the work by Zeigler et al. (1961) 1,000 p.p.m. of zinc appeared to depress the growth of chicks at 26 days of age slightly but did not increase mortality. The estimated zinc requirements of chicks and turkey poults, given in the NAS-NRC "Nutrient Requirements for Poultry" (1960b) are 45 p.p.m. and 55 p.p.m., respectively (Fig. 6.4).

Fluorine. No satisfactory evidence that fluorine is an essential mineral has yet been obtained. McClendon and Gershon-Cohen (1953) reported retarded growth and increase in dental caries in rats from fluorine deficiency but no one yet has been able to confirm these results. The value of adding 1.0–1.2 p.p.m. of fluorine to the drinking water for the prevention of dental caries in children, however, has been demonstrated.

When appreciable quantities of fluorine are ingested by animals, harmful effects result. These may not be revealed immediately as the toxic effects of fluorine are

FIG. 6.4 — Enlarged hocks in a turkey poult caused by zinc deficiency.

cumulative. The fluorine tolerance of chickens seems to be definitely higher than that of other species of farm animals.

Halpin and Lamb (1932) showed that the toxic level of fluorine in the rations of chickens is between 0.035 and 0.070 per cent. Excess fluorine resulted in retarded growth in young chicks and in lower egg production and loss of weight in laying hens. On the other hand, Hauck et al. (1933) obtained evidence that the toxic level of fluorine, as measured by the growth of chicks, lies between 0.068 and 0.136 per cent. Kick et al. (1933) reported that the toxic level is between 0.036 and 0.072 per cent. On feeding graded levels of fluorine to turkey poults eight weeks of age, Anderson et al. (1955) found that up to 400 p.p.m. did not affect weight gains, feed consumption, or feed efficiency during the subsequent eight weeks.

The Association of American Feed Control Officials (1953) has recommended

that the fluorine content of minerals or mineral mixtures used in poultry feedstuffs contain not more than 0.60 per cent of fluorine, and that the total fluorine content of poultry rations should not exceed 0.035 per cent. The Subcommittee on Fluorosis Problems of the NAS-NRC Committee on Animal Nutrition (NAS-NRC, 1960a) has suggested that the level of fluorine be limited to 0.015–0.03 per cent of the ration for chickens, and 0.03–0.04 per cent for turkeys when the fluorine is supplied by soluble fluorides. The quantity in chicken rations may be increased to 0.03–0.04 per cent when the fluorine is supplied by phosphatic limestone or rock phosphate.

The fluorine hazard in poultry feeding, outside of certain local areas, is related largely to the use of fluorine-containing mineral supplements. These minerals are the different varieties of phosphate rock, the superphosphates produced from them, and the phosphatic limestones. Raw rock phosphates ordinarily contain from 3.25 to 4.0 per cent fluorine, while superphosphate and phosphatic limestone usually contain appreciably less. Heat treatment of the raw rock phosphate or superphosphate is employed to remove the fluorine from these products. Commercially defluorinated phosphate rock and superphosphate as now marketed generally contain as little as 0.02 to 0.03 per cent of fluorine and come well within the limits of permissible fluorine content.

Selenium. Selenium has been shown to be an essential mineral element for the chick in work conducted independently by Scott et al. (1957) and Patterson et al. (1957). Selenium was found to prevent the development of exudative diathesis in chicks fed a Torula yeast diet deficient in vitamin E. Under these conditions Patterson et al. reported that as little as 0.3 p.p.m. of selenium gave complete protection against exudative diathesis, while Scott et al. observed that a level of 0.1 p.p.m. was effective in preventing the development of this syndrome

Norris et al. (1957) reported that no growth stimulation or other effects from selenium were obtained in chicks fed a purified soybean protein diet containing adequate amounts of vitamin E. Because of these findings, the inclusion of selenium in chick rations seems unnecessary and perhaps undesirable in view of evidence obtained by Poley et al. (1941) and Carlson et al. (1954) that 10 or more p.p.m. is toxic for the chick.

This quantity of selenium is also toxic to breeders. Poley and Moxon (1938) fed laying rations containing 2.5, 5.0, and 10.0 p.p.m. of selenium. At the intermediate level, hatchability was not appreciably affected but some chicks had wiry down. The high level, however, reduced the hatchability to zero, the dead embryos showing short upper beaks, edema of head and neck, missing toes and eyes, wiry down, and other characteristics of selenium poisoning. No effects on body weight, egg production, or fertility were observed. Moxon and Poley (1938) showed that the selenium content of the body of hens and of eggs increased proportionally to the amount in the ration.

Molybdenum. Higgins et al. (1956) obtained evidence, by resorting to the use of tungsten as a molybdenum antagonist, that this mineral element is required for the growth of chicks as well as for the maintenance of the xanthine oxidase content of chick tissues. Reid et al. (1956) also reported that molybdenum stimulates growth of chicks and poults. Purified diets were used in the experimental work of these investigators. Norris et al. (1957) and Leach et al. (1962) confirmed the results of Higgins et al. (1956) using tungsten as an antagonist. Without the inclusion of tungsten in the purified diet, a significant growth increase of 6.1 per cent was obtained by adding molybdenum to a chick diet containing isolated soybean protein and 4.8 p.p.m. of zinc. Increasing the zinc to 64.8 p.p.m. or replacing the isolated soybean protein with casein resulted in no growth increase. The requirement of the

TABLE 6.6

APPROXIMATE ENERGY CONTENT AND AVERAGE COMPOSITION OF COMMON POULTRY FEEDSTUFFS*

Material	Metabolizable Energy (kcal/lb)	Productive Energy (kcal/lb)	Protein (%)	Fat (%)	N-free Extract (%)	Fiber (%)	Mineral Matter (%)	Calcium (%)	Phosphorus (%)	Iron (mg/lb)	Copper (mg/lb)	Manganese (mg/lb)	Zinc (mg/lb)
Alfalfa leaf meal, 20%	720	310	20.9	2.9	38.2	19.2	8.8	1.7	0.2	149.8	3.1	15.0	9.1
Alfalfa meal, 17%	720	250	17.8	1.8	39.7	24.2	8.8	1.4	0.2				
Barley, Pacific Coast	1,280	800	12.7	1.9	66.6	5.4	2.8	0.09	0.3	31.8	5.0	8.9	7.8
Barley, except Pacific Coast	1,280	800	9.7	2.2	68.4	6.2	2.2	0.09	0.3	38.1	7.4	7.6	7.7
Bone meal, steamed			12.1	1.2		1.0	79.6	28.34	13.59	381.4		13.8	193.0
Buttermilk, dried	1,160	520	33.0	5.7	47.7	0.7	7.6	1.34	0.94			1.6	2.8
Corn, whole	1,330	1,100	8.9	3.9	68.9	2.0	1.3	0.02	0.3	9.1	0.9	2.3	11.8
Corn distillers dried solubles	1,350	850	29.5	9.1	45.9	4.6	7.0	0.35	1.37	250.6	37.6	33.4	45.9
Corn gluten meal	1,350	840	43.0	2.1	39.6	4.1	2.4	0.16	0.9	181.1	12.8	3.3	31.3
Cottonseed meal	790		40.6	6.1		10.1		0.20	1.09	119.4		9.3	
Fat, stabilized	3,500	2,900		99.7									
Fish meal, menhaden	1,320	900	61.5	7.7	2.9	0.7	19.6	5.49	2.81	234.2	3.8	11.7	46.5
Fish meal, sardine	1,320	900	65.5	6.9	6.9	0.8	15.7	4.9	2.77	135.3	9.2	10.1	
Fish solubles	650	450	31.4	4.5	2.0	0.6	16.1	0.61	1.35	155.3	21.4	5.4	15.4
Hominy feed, yellow	1,310	860	11.1	6.5	64.9	4.8	2.9	0.66	0.61		44.1	3.3	
Liver and glandular meal	1,720	720	55.1	15.0	2.6	1.6	9.1	1.57	5.07	222.5	0.7	5.6	75.0
Meat and bone scrap, 50%	900	720	50.5	8.3	2.6	2.2	29.1	10.57	5.07	225.6	4.4	3.5	75.0
Meat scrap, 55%	900	720	53.4	8.9	2.6	2.2	25.2	7.94	4.03	199.3	4.4	4.3	
Milk, including skim	1,160	520	13.0	0.9	51.7	0.2	7.6	1.24	0.45	23.6	2.4	1.0	2.8
Oats, Pacific Coast	1,210	740	12.0	5.4	58.1	11.0	4.0	0.09	0.4	36.3		19.2	
Oats, except Pacific Coast	1,210	760	9.0	4.7	61.1	11.0	3.0	0.07	0.35		1.6		
Oatmeal, feeding	1,540		16.1	6.3	63.4	1.9	2.2	0.03	0.5	22.6		18.7	
Peanut meal	1,500		41.3	2.9	26.0	9.2	2.2	0.03	0.5	122.6	7.8	5.9	7.0
Sorghum, milo	1,500		11.3	2.9	71.3	2.0	1.7	0.03	0.3	22.7			
Soybean meal, 50%	1,140	610	50.9	0.8	29.7	2.8	5.6	0.26	0.62	76.7	8.0	20.7	33.2
Soybean meal, 44%	1,070	570	44.7	1.0	30.8	7.0	5.7	0.29	0.61		2.0	18.0	32.7
Wheat, hard red, winter	1,400	920	15.7	1.9	68.3	2.7	1.9	0.05	0.3	24.2	4.4	27.7	15.9
Wheat, Pacific Coast	1,400	920	9.9	2.0	72.7	2.7	6.1	0.14	0.4	31.8	5.6	52.6	
Wheat, soft, Pacific Coast	1,400	920	9.0	1.9	73.2	2.2	4.9	0.15	0.17			53.8	
Wheat bran	590	580	16.0	4.1	55.9	9.6	6.1	0.14	0.91	47.2	10.2	27.7	
Wheat standard middlings	860		17.2	4.1	55.9	9.6	4.9						
Wheat, red dog	1,240		18.0	3.6	62.6	2.3	2.5	1.08	0.52	27.7	2.0	17.1	3.4
Whey, dried	870	490	13.1	1.1	59.8	0.2	14.0	1.65	0.96		15.0	1.6	22.2
Yeast, dried brewers	920	480	44.6	2.5	38.6	2.2	7.3	0.13	1.43	58.1			

* The data on metabolizable energy were obtained for the most part from a compilation by F. W. Hill, University of California, Davis, April, 1963, those on productive energy for the most part from the report by Fraps (1946), and the information on feed composition from various sources, including NAS-NRC Pub 659, 1959

chick for molybdenum when fed the casein diet was found to be less than 0.11 p.p.m.

Amounts of molybdenum of 100 p.p.m. or more have been shown by Arthur *et al.* (1958) to cause growth depression in chicks fed a practical diet containing 7.0 p.p.m. The depression was partially overcome at 200 and 350 p.p.m. of molybdenum by addition of copper but not at 500 p.p.m. Davies *et al.* (1957) observed marked growth depression in chicks at 500 p.p.m. of molybdenum which was partially overcome by supplementation with sodium sulfate. These investigators found an anemic condition associated with levels of molybdenum in excess of 2,000 p.p.m. Kratzer (1952) partially overcame growth depression in turkey poults as well as chicks, due to high levels of molybdenum, with added copper.

WATER

The simple chemical compound, water, is of unequalled importance in the metabolism of all animals. Water holds this unique position in nutrition mainly because of its physical properties. Due to its solvent and polar properties, it acts as a transport medium for all other nutrients and products of metabolism and enhances cell reactions. Because of its high specific heat it can absorb the heat of reaction produced in the burning of carbohydrates and fats with little rise in temperature. Water evaporates readily, removing many calories of heat from the body as latent heat of vaporization. These and the many other functions of water make it evident why the animal body is able to exist much longer without food than it can without water.

Importance of a continuous water supply for poultry. Unlike larger farm animals, chickens must have access to a continuous water supply since they drink only a small amount at a time. An insufficient amount of water results in decreased growth and egg production. During severely cold weather it is also necessary to make some provision which will keep the water warm. According to Beresford (1930), failure to warm the drinking water for hens during cold weather results in lowered water consumption and reduced egg production. Hammond (1944) has observed that a lack of water causes the development of loose, slimy gizzard linings which accompany early, nonspecific mortality in turkey poults.

The quantity of water needed by chickens has been found to be related to the amount of some of the nutrients in the diet. Heuser (1952) reported results in confirmation of previous observations which showed that the quantity of water drunk by chicks is correlated directly with the salt content of the diet. Wheeler and James (1950) observed a similar correlation with protein content. Both increased water elimination, the former by increasing the water content of the feces, and the latter by increasing the total fecal excretion. The latter investigators also found that increasing the quantity of soybean meal in the ration increased water intake and elimination.

ENERGY CONTENT AND COMPOSITION OF FEEDSTUFFS

The metabolizable and productive energy content and the amount of protein, fat, fiber, nitrogen-free extract, and mineral matter in the common poultry feedstuffs are given in Table 6.6. In addition, values for calcium and phosphorus content and several of the trace elements are included in the table. Values for vitamin content are given in the chapter on vitamins. With this exception the data in this table, together with the data given in previous ones, provide the basic information needed in the formulation of satisfactory poultry rations.

REFERENCES

Ackerson, C. W., Blish, M. J., and Mussehl, F. E.: 1939. The utilization of food elements by growing chicks. 6. The influence of the protein level of the ration on the growth of chicks. Nebr. Agr. Exper. Sta., Res. Bul. 108.

Alder, B.: 1927. The use of calcite and other natural deposits of calcium carbonate in the ration of laying hens. Proc. Third World's Poultry Cong., p. 231.

Almquist, H. J.: 1942. Magnesium requirement of the chick. Proc. Soc. Exper. Biol. and Med. 49: 544.

————: 1947. Evaluation of amino acid requirements by observations on the chick. Jour. Nutr. 34:543.

————: 1952. Amino acid requirements of chickens and turkeys. A review. Poultry Sci. 31:966.

————: 1957. Proteins and Amino Acids in Animal Nutrition. 4th ed. Reprinted courtesy U. S. Ind. Chem. Co., New York.

————, and Asmundson, V. S.: 1944. High protein mashes for broilers. Poultry Sci. 23:67.

————, Mecchi, E., Kratzer, F. H., and Grau, C. R.: 1942. Soybean protein as a source of amino acids for the chick. Jour. Nutr. 24:385.

Anderson, J. O., Hurst, J. S., Strong, D. C., Nielsen, N. A., Greenwood, D. A., Robinson, W., Shupe, J. L., Binns, W., Bagley, R. A., and Draper, C. I.: 1955. Effect of feeding various levels of sodium fluoride to growing turkeys. Poultry Sci. 34:1147.

Anonymous: 1955. Chicken raising experiments. Queensland Agr. Jour. 44:425.

Arthur, D., Motzok, I., and Branion, H. D.: 1958. Interaction of dietary copper and molybdenum in rations fed to poultry. Poultry Sci. 37:1181.

Association of American Feed Control Officials, Inc.: 1953. Official Publication, p. 33.

Atkinson, R. L., Kurnick, A. A., Ferguson, T. M., Reid, B. L., Quisenberry, J. H., and Couch, J. R.: 1957. Protein and energy levels for turkey starting diets. Poultry Sci. 36:767.

Barlow, J. S., Slinger, S. J., and Zimmer, R. P.: 1948. The reaction of growing chicks to diets varying in sodium chloride content. Poultry Sci. 27:542.

Ben Dor, Ben-Ami: 1941. Requirements of potassium by the chick. Proc. Soc. Exper. Biol. and Med. 46:341.

Beresford, H.: 1930. Stock tank and poultry water heaters. Agr. Eng. 11:279.

Bethke, R. M., Kennard, D. C., and Kick, C. H.: 1929a. The availability of calcium in calcium salts and minerals for bone formation in the growing chick. Poultry Sci. 9:45.

————, Kennard, D. C., Kick, C. H., and Zinzalian, G.: 1929b. The calcium-phosphorus relationship in the nutrition of the growing chick. Poultry Sci. 8.257.

Bird, H. R., Rubin, M., Whitson, D., and Haynes, S. K.: 1946. Effectiveness of dietary supplements in increasing hatchability of eggs and viability of progeny of hens fed a diet containing a high level of soybean oil meal. Poultry Sci. 25:285.

Boatner, C. H., and Hall, C. M.: 1946. Pigment glands of cottonseed. 1. Behavior of the glands toward organic solvents. Oil and Soap 23:123.

Borchers, R., and Ackerson, C. W.: 1950. The nutritive value of legume seeds. X. Effect of autoclaving and the trypsin inhibitor test for 17 species. Jour. Nutr. 41:339.

————, Ackerson, C. W., and Mussehl, F. E.: 1948. Trypsin inhibitor. VIII. Growth inhibiting properties of a soybean trypsin inhibitor. Arch. Biochem. 19.317.

Briggs, G. M., Groschke, A. C., and Lillie, R. J.: 1946. Effect of proteins low in tryptophane on growth of chickens and on laying hens receiving nicotinic acid-low rations. Jour. Nutr. 32.659.

Bronkhorst, J. J.: 1938. The influence of the protein level of diet on the growth, egg production, egg weight and mortality of Single Comb White Leghorn pullets. Onderstepoort Vet. Sci. 10.469.

Buckner, G. D., Martin, J. H., and Insko, W. M., Jr.: 1932. The effect of magnesium carbonate when added to diets of growing chicks. Poultry Sci. 11:58.

Burns, C. H., Cravens, W. W., and Phillips, P. H.: 1952. The requirement of breeding hens for sodium chloride. Poultry Sci. 31:302.

————, Cravens, W. W., and Phillips, P. H.: 1953. The sodium and potassium requirements of the chick and their interrelationship. Jour. Nutr. 50 317.

Burr, G. O., and Burr, M. M.: 1930. On the nature and role of the fatty acids essential in nutrition. Jour Biol Chem. 86 587.

————, Burr, M. M., and Miller, E. S.: 1932. On the fatty acids essential in nutrition. III. Jour. Biol. Chem. 97:1.

Byerly, T. C.: 1941. Feed and other costs of producing market eggs. Univ. Maryland Agr. Exper. Sta., Bul. A1 (Technical).

————, Titus, H. W., and Ellis, N. R.: 1933. Production and hatchability of eggs as affected by different kinds and quantities of proteins in the diet of laying hens. Jour. Agr. Res. 46:1.

Carlson, C. W., Guenthner, E., Kohlmeyer, W., and Olson, O. E.: 1954. Some effects of selenium, arsenicals and vitamin B_{12} on chick growth. Poultry Sci. 33.768.

Carver, J. S., Heiman, V., Cook, J. W., and St. John, J. L.: 1939. The protein requirements of White Leghorn pullets. Wash. Agr. Exper. Sta., Bul. 383

————, St. John, J. L., Aspinall, T. E., and Flor, I. H.: 1932. Protein requirements of chickens. Poultry Sci. 11:45.

Caskey, C. D., Gallup, W. D., and Norris, L. C.: 1939. The need for manganese in the bone development of the chick. Jour. Nutr. 17:407.

————, and Norris, L. C.: 1938. Further studies on the role of manganese in poultry nutrition. Poultry Sci. 17:433.

————, and Norris, L. C.: 1940. Micromelia in adult fowl caused by manganese deficiency during embryonic development. Proc. Soc. Exper. Biol. and Med. 44 332.

————, Norris, L. C., and Heuser, G. F.: 1944. A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poultry Sci. 23:516.

Combs, G. F., and Romoser, G. L.: 1955. A new approach to poultry feed formulation. Maryland Agr. Exper. Sta. Misc. Publ. No. 226.

————, Norris, L. C., and Heuser, G. F.: 1942. The interrelationship of manganese, phosphatase, and vitamin D in bone development. Jour. Nutr. 23:131.

Common, R. H.: 1932. Mineral balance studies on poultry. Jour. Agr. Sci. 22:576.

————: 1940. Observations on the mineral metabolism of pullets. IV. Jour. Agr. Sci. 30:113.

Couch, J. R., James, L. E., and Sherwood, R. M.: 1947. The effect of different levels of manganese and different amounts of vitamin D in the diet of hens and of pullets. Poultry Sci 26:30

Creek, R. D., Parker, H. E., Hauge, S. M., Andrews, F. N., and Carrick, C. W.: 1957. The iodine requirements of young chickens. Poultry Sci. 36.1360.

————, Parker, H. E., Hauge, S. M., Andrews, F. N., and Carrick, C. W.: 1960. The influence of body weight on the experimental production of perosis by manganese deficiency. Poultry Sci 39:96.

Davies, R. E., Kurnick, A. A., Reid, B. L., and Couch, J. R.: 1957. Molybdenum and sulfate interaction in the nutrition of the growing chick. Poultry Sci 36-1111.

Davis, P. N., Norris, L. C., and Kratzer, F. H.: 1962. Interference of soybean proteins with the utilization of trace minerals. Jour. Nutr. 77:217.

————, Norris, L. C., and Kratzer, F. H.: 1962. Iron deficiency studies in chicks using treated isolated soybean protein diets. Jour. Nutr. 78:415.

Davis, R. L., Briggs, G. M., and Sloan, H. J.: 1953. Effect of cobalt on diet of the chick. Proc Soc. Exper. Biol. and Med. 82:175.

Day, E. J., and Hill, J. E.: 1957. The effect of calorie protein ratio of the ration on growth and feed efficiency of turkeys. Poultry Sci. 36:773.

Donaldson, W. E., Combs, G. F., and Romoser, G. L.: 1956. Studies on energy levels in poultry rations. I. The effect of calorie protein ratio of the ration on growth, nutrient utilization and body composition of chicks. Poultry Sci. 35:1100

Edwards, H. M., Jr., Norris, L. C., and Heuser, G. F.: 1956. Studies on the lysine requirement of chicks. Poultry Sci. 35:585.

————, Young, R. J., and Gillis, M. B.: 1958. Studies on zinc in poultry nutrition. I. The effect of feed, water and environment on zinc deficiency in chicks. Poultry Sci. 37:1094.

Elvehjem, C. A., Hart, E. B., and Kemmerer, A. R.: 1929. The relation of iron and copper to hemoglobin synthesis in the chick. Jour. Biol. Chem. 84:131.

Erikson, S. E., Boyden, R. E., Martin, J. H., and Insko, W. M., Jr.: 1933. The iron and copper content of egg yolk. Ky. Agr. Exper. Sta., Bul. 342:155.

Evans, R. J., and Butts, H. A.: 1948. Studies on the heat inactivation of lysine in soybean oil meal. Jour. Biol. Chem. 175:15.

————, and Carver, J. S.: 1941. The effect of mineral metabolism on egg shell quality. Wash. Agr. Exper. Sta., Bul. 410:92.

————, Carver, J. S., and Brant, A. W.: 1944a. The influence of dietary factors on egg shell quality. I. Phosphorus. Poultry Sci. 23:9.

————, Carver, J. S., and Brant, A. W.: 1944b. The influence of dietary factors on egg shell quality. II. Calcium. Poultry Sci. 23:56.

————, McGinnis, J., and St. John, J. L.: 1947. The influence of autoclaving soybean oil meal on the digestibility of the proteins. Jour. Nutr. 33.661.

Ferguson, T. M., Vaught, H. P., Matterson, L. D. Reid, B. L., and Couch, J. R.: 1957. The effect of different levels of productive energy, protein and methionine upon the growth of Broad Breasted Bronze turkey poults. Poultry Sci. 36:124.

Fraps, C. S.: 1946. Composition and productive energy of poultry feeds and rations. Tex. Agr Exper. Sta., Bul. 678.

Fritz, J. C., Halpin, J. L., and Hooper, J. H.: 1947. Studies on the nutritional requirements of poults. Poultry Sci. 26-78.

————, Hooper, J. H., Halpin, J. L., and Moore, H. P.: 1916. Failure of feather pigmentation in bronze poults due to lysine deficiency. Jour. Nutr. 31:387.

————, and Margolf, P. H.: 1932. Feed consumption and costs in raising turkeys. Pa. Agr. Exper. Sta., Bul. 250.

Funk, E. M., and Margolf, P. H.: 1937. Studies on the importance of manganese in the nutrition of poultry. Poultry Sci. 16:331.

Gallup, W. D., and Norris, L. C.: 1938. The essentialness of manganese for the normal development of bone. Science 87:18.

————, and Norris, L. C.: 1939a. The amount of manganese required to prevent perosis in the chick. Poultry Sci. 18:76.

————, and Norris, L. C.: 1939b. The effect of a deficiency of manganese in the diet of the hen. Poultry Sci. 18 83

Gardiner, E. E., Rogler, J. C., and Parker, H. E.: 1959. Magnesium requirement of the chick. Poultry Sci. 58·1207.

Gassner, F. X., and Wilgus, H. S., Jr.: 1940. Congenital goiter in chicks. Poultry Sci. 19:349.

Gericke, A. M., and Platt, C. S.: 1932. Feather development in Barred Plymouth Rock chicks. N.J. Agr. Exper. Sta., Bul. 543.

Gillis, M. B.: 1948. Potassium requirement of the chick. Jour. Nutr. 36:351.

———. 1950. Further studies on the role of potassium in growth and bone formation. Jour. Nutr. 42:45.

———, Norris, L. C., and Heuser, G. F.: 1949. The effect of phytin on the phosphorus requirement of the chick. Poultry Sci. 28:283.

———, Norris, L. C., and Heuser, G. F.: 1953. Phosphorus metabolism and requirements of hens. Poultry Sci. 32:977.

Godfrey, P. R., Carrick, C. W., and Quackenbush, F. W.: 1953. Iodine nutrition of chicks. Poultry Sci. 32:394.

Golding, W. V., Schaible, P. J., and Davidson, J. A.: 1940. A breed difference in the manganese requirement of laying hens. Poultry Sci. 19:263.

Gordon, R. S., and Sizer, I. W.: 1955. Ability of sodium sulfate to stimulate growth of the chicken. Science 122:1270.

Grau, C. R., and Kamei, M.: 1950. Amino acid imbalance and the growth requirements for lysine and methionine. Jour. Nutr. 41:89.

———, Kratzer, F. H., and Asmundson, V. S.: 1946. The lysine requirements of poults and chicks. Poultry Sci. 25:529.

Greene, D. E., Scott, H. M., and Johnson, B.C.: 1960. A need for glycine in crystalline amino acid diets. Poultry Sci. 39:512.

Croschke, A. C., Rubin, M., and Bird, H. R.: 1947. Gland-free cottonseed meal as a protein supplement for chickens. Poultry Sci. 26:310.

Cutowska, M. S., and Parkhurst, R. T.: 1942. Studies in mineral nutrition of laying hens. II. Excess of calcium in the diet. Poultry Sci. 21:321.

Halnan, E. T.: 1925. The calcium, phosphorus and nitrogen balance of the nonlaying and laying pullet. Jour. Nat. Poultry Inst. 10:410.

———. 1936. The role of minerals in poultry nutrition. Proc. Sixth World's Poultry Cong. 1:53.

Halpin, J. G., Holmes, C. E., and Hart, E. B.: 1934. Salt requirements of growing chicks. Poultry Sci. 13:308.

———, and Lamb, A. R.: 1932. The effect of ground phosphate rock fed at various levels on the growth of chicks and on egg production. Poultry Sci. 11:5.

Hamlyn, W. L., Branion, H. D., and Cavers, J. R.: 1934. The influence of protein on the growth of ducks. Poultry Sci. 13:333.

Hammond, J. C.: 1944. Lack of water a cause of loose, slimy gizzard linings accompanying early mortality in poults. Poultry Sci. 23.477.

———, McClure, H. E., and Kellogg, W. L.: 1944. The minimum phosphorus requirements of growing turkeys. Poultry Sci. 23:259.

Hart, E. B., Scott, H. T., Kline, O. L., and Halpin, J. C.: 1930. The calcium-phosphorus ratio in the nutrition of the growing chick. Poultry Sci. 9.296

Hartwigk, H.: 1931. Ueber das Vorkommen der Visceralgicht (Eingeweidegicht) bei Jungbühnern und Hühnerkuken. Tierärztl. Rundschau 37.812

Hauck, H. M., Steenbock, H., Lowe, J. T., and Halpin, J. G.: 1933. Effect of fluorine on growth, calcification and parathyroids in the chicken. Poultry Sci. 12:242.

Hayward, J. W., and Hafner, F. H.: 1941. The supplementary effect of cystine and methionine upon the protein of raw and cooked soybeans as determined with chicks and rats. Poultry Sci. 20:139.

Headley, F. B., and Knight, E. W.: 1938 Turkey feeding experiments. Nev. Agr. Exper. Sta., Bul. 148.

Heiman, V., Carver, J. S., and St. John, J. L.: 1936. The protein requirement of laying hens. Wash. Agr. Exper. Sta., Bul. 351.

Henneberg, W., and Stohmann, F.: 1860, 1865. Beiträge zur Begründung einer rationellen Futterung der Wiederkäuer. I, II, Schwetschke u Sohn, Brunswick.

Heuser, G. F.: 1956. The protein requirements of laying hens. Proc. Sixth World's Poultry Cong. 1:276

———. 1941. Protein in poultry nutrition—a review. Poultry Sci. 20:362.

———. 1952. Salt additions to chick rations. Poultry Sci. 31.85.

———, and Norris, L. C.: 1933. The influence of the protein level on the growth of chickens and its relation to subsequent behavior. Proc. Fifth World's Poultry Cong. II-551.

———, Norris, L. C., and McGinnis, J.: 1946. Vegetable protein concentrates fed alone and in combination with soybean oil meal and fish meal as the chief supplementary protein in chick starting rations. Poultry Sci. 25:138

———, Norris, L. C., Peeler, H. T., and Scott, M. L.: 1945. Further studies on the apparent effect of digestibility upon growth, weight maintenance and egg production. Poultry Sci. 24:142.

Higgins, E. S., Richert, D. A., and Westerfeld, W. W.: 1956. Molybdenum deficiency and tungstate inhibition studies. Jour. Nutr. 59 539.

Hill, C. H., and Matrone, G.: 1961. Studies on copper and iron deficiencies in growing chickens. Jour. Nutr. 73:425.

Hill, D. C.: 1944. Protein in poultry nutrition. Scient. Agr 24:551.

Hill, F. W.: 1949. Studies of the protein requirement of chicks. Proc. Cornell Nutr. Conf. for Feed Manufacturers, p. 57.

———: 1958. New viewpoints in the nutrition of layers. Feedstuffs 30 (No. 45):40, 41, 44.

———, and Anderson, D. L.: 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. Jour. Nutr. 64:587.

———, Anderson, D. L., Renner, R., and Carew, L. B., Jr.: 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poultry Sci. 39:573.

———, and Dansky, L. M.: 1954. Studies on the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. Poultry Sci. 33:112.

———, and Renner, R.: 1957. Metabolizable energy values of feedstuffs for poultry and their use in formulation of rations. Proc. Cornell Nutr. Conf. for Feed Manufacturers, p. 22.

———, and Renner, R.: 1960. The metabolizable energy of soybean oil meals, soybean mill feeds and soybean hulls for the growing chick. Poultry Sci. 39:579.

Hopkins, D. T., and Nesheim, M. C.: 1962. Further studies on linoleic acid deficiency in hens and chicks. Proc. Cornell Nutr. Conf. for Feed Manufacturers, p. 104.

———, Nesheim, M. C., Carew, L. B., Jr., and Norris, L. C.: 1960. Unsaturated fatty acids in poultry nutrition. Proc. Cornell Nutr. Conf. for Feed Manufacturers, p. 71.

Horton, D. H.: 1952. A comparison of feeding a twelve per cent and a nineteen per cent protein ration to White Pekin ducklings. Poultry Sci. 11:106.

Hull, T. A., and Rettger, L.: 1917. Influence of milk and carbohydrate feeding on the character of intestinal flora. Jour. Bact. 2:47.

Hurwitz, S., and Griminger, P.: 1960. Observations on the calcium balance of laying hens Jour. Agr. Sci. 54:373.

Insko, W. M., Jr., Lyons, M., and Martin, J. H.: 1938. The quantitative requirement of the growing chick for manganese. Jour. Nutr. 15:621.

Jensen, L. S., Allred, J. B., Fry, R. E., and McGinnis, J.: 1958. Evidence for an unidentified factor necessary for maximum egg weight in chickens. Jour. Nutr. 65:219.

———, and McGinnis, J.: 1961. Nutritional investigations with turkey hens. 1. Quantitative requirement for protein. Poultry Sci. 40:288.

Johnson, B. J., and Fisher, H.: 1959. The amino acid requirement of laying hens. 3. Minimal requirement levels of essential amino-acids: techniques and development of diet. Brit. Jour. Nutr. 12:276.

Keene, O. D., and Combs, G. F.: 1962. Magnesium requirement of chicks and poults. Poultry Sci. 41:1654.

Kick, C. H., Bethke, R. M., and Record, P. R.: 1933. Effect of fluorine in the nutrition of the chick. Poultry Sci. 12:382.

Klain, G. J., Scott, H. M., and Johnson, B. C.: 1960. The amino acid requirement of the growing chick fed a crystalline amino acid diet. Poultry Sci. 39:39.

Kratzer, F. H.: 1952. Effect of dietary molybdenum upon chicks and poults. Proc. Soc. Exper. Biol. and Med. 80:483.

———, Allred, J. B., Davis, P. N., Marshall, B. J., and Vohra, P.: 1959. The effect of autoclaving soybean protein and the addition of ethylenediaminetetraacetic acid on the biological availability of dietary zinc for turkey poults. Jour. Nutr. 68:313.

Leath, R. M., Jr., Dam, R., Zeigler, T. R., and Norris, L. C.: 1959. The effect of protein and energy on the potassium requirement of the chick. Jour. Nutr. 81:193.

———, and Nesheim, M. C.: 1963. Studies on chloride deficiency in chicks. Jour. Nutr. 81:193.

———, Turk, D. E., Zeigler, T. R., and Norris, L. C.: 1962. Studies on the role of molybdenum in chick nutrition. Poultry Sci. 41:300.

———, Zeigler, T. R., and Norris, L. C.: 1958. The potassium requirement of broiler chicks fed a high energy diet. (Unpub. results, Cornell Univ. Agr. Exper. Sta.)

Lloyd, M. D., Reed, C. A., and Fritz, J. C.: 1949. Experiences with high protein diets for chicks and poults. Poultry Sci. 28:69.

Lyons, M.: 1939. Some effects of manganese on eggshell quality. Ark. Agr. Exper. Sta., Bul. 374

———, and Insko, W. M., Jr.: 1937. Chondrodystrophy in the chick embryo produced by manganese deficiency in the diet of the hen. Ky. Agr. Exper. Sta., Bul. 371

McClendon, J. F., and Gershon-Cohen, J.: 1953. Trace element deficiencies. Water culture crops designed to study deficiencies in animals. Jour. Agr. and Food Chem. 1:464.

McConathie, J. D., Graham, W. R., Jr., and Branion, H. D.: 1955. A study of the protein requirements of growing chicks. Scient. Agr. 15:751.

McGinnis, J.: 1944. Studies on the utilization by the chick of phosphorus supplied entirely from plant sources. Thesis, Cornell Univ.

———, Norris, L. G., and Heuser, G. F.: 1944. Poor utilization of phosphorus in cereals and legumes by chicks for bone development. Poultry Sci. 23:157.

———: 1961. Magnesium requirement of the chick determined with a magnesium-free diet. Poultry Sci 40:1174.

McWard, G. W., and Scott, H. M.: 1961. Magnesium requirement of the chick determined with a magnesium-free diet. Poultry Sci 40:1174.

Machlin, L. J.: 1955. Studies on the growth response in the chicken from the addition of sulfate to a low sulfur diet. Poultry Sci 34:1209.

————, and Dudley, W. A.: 1962. Effect of linoleic acid and methylmyristate on egg production and hatchability in the laying hen. Poultry Sci. 41:1659.

Machlin, L. J., and Gordon, R. S.: 1961. Effect of dietary fatty acids and cholesterol on growth and fatty acid composition of the chicken. Jour. Nutr. 75:157.

Margolf, P. H.: 1929. The effects of various protein-carbohydrate ratios upon the mortality, growth and condition of Single Comb White Leghorn chicks. 42nd Ann. Rep. Pa. St. Coll., Pa. Agr. Expt. Sta., Bul. 243:28.

Marion, J. E., and Edwards, H. M., Jr.: 1962. The response of fat deficient laying hens to corn oil supplementation. Poultry Sci. 41:1785.

Marlow, H. W., and King, H. H.: 1936. Sulfur in eggs. Poultry Sci. 15:377.

Matterson, L. D., Scott, H. M., and Jungherr, E.: 1946. Salt tolerance of turkeys. Poultry Sci. 25,539.

Mattill, H. A.: 1927. The oxidative destruction of vitamins A and E and the protective action of certain vegetable oils. Jour. Am. Med. Assn. 89:1505.

Mayall, G.: 1929. Visceral gout in poultry. Vet. Jour. 85:230.

Mayo, R. H., Hauge, S. M., Parker, H. E., Andrews, F. N., and Carrick, C. W.: 1956. Copper tolerance of young chickens. Poultry Sci. 35,1156.

Melnick, D., Oser, B. L., and Weiss, S.: 1946. Rate of enzymic digestion of proteins as a factor in nutrition. Science 103:326.

Menge, H., and Denton, C. A.: 1961. Effect of dried egg yolk, oils and fat on chick growth. Jour. Nutr. 75:107.

Miller, E. C., Sunde, M. L., and Elvehjem, C. A.: 1957. Minimum protein requirement of laying pullets at different energy levels. Poultry Sci. 36 681.

Miller, M. W., and Borse, G. E.: 1934. Phosphorus requirements of laying hens. Wash. Agr. Exper. Sta., Bul. 306.

Miller, R. F., Small, G., and Norris, L. C.: 1955. Studies on the effect of sodium bisulfite on the stability of vitamin E. Jour. Nutr. 55:81.

Milne, H. I.: 1932. Protein requirements of growing chicks. Scient. Agr. 12:604.

Mitchell, H. H., Card, L. E., and Carman, G. G.: 1926b. The toxicity of salt for chickens. Ill. Agr. Expert Sta., Bul. 279.

————, Card, L. E., and Hamilton, T. S.: 1926a. The growth of White Plymouth Rock chickens. Ill. Agr. Exper. Sta., Bul. 278.

————, Card, L. E., and Hamilton, T. S.: 1931. A technical study of the growth of White Leghorn chickens. Ill. Agr. Exper. Sta., Bul. 567:83.

————, and Carman, G. G.: 1926. Does the addition of sodium chloride increase the value of a corn ration for growing animals? Jour. Biol. Chem. 68.165.

Morris, L., Thompson, R. B., and Heller, V. G.: 1932. The effect of varying the amounts of protein in the poultry ration on chick growth and subsequent egg production. Poultry Sci. 11,364.

Motzok, I., and Slinger, S. J.: 1948. Studies on the calcium and phosphorus requirements of Broad Breasted Bronze turkeys. Poultry Sci. 27:486.

Moxon, A. L., and Poley, W. E.: 1938. The relation of selenium content of grains in the ration to the selenium content of poultry carcass and eggs. Poultry Sci. 17:77.

Mussell, F. E., and Ackerson, C. W.: 1935. Calcium and phosphorus requirements of growing turkeys. Poultry Sci. 14:147.

————, Ackerson, C. W., and Thayer, R. H.: 1941. Protein utilization by the growing poult. Poultry Sci. 20·469.

————, Hill, R. S., Blish, M. J., and Ackerson, C. W.: 1930. Utilization of calcium by the growing chick. Jour. Agr. Res. 40:191.

National Academy of Sciences-National Research Council: 1959. Joint United States-Canadian tables of feed composition. Pub. 659.

————: 1960a. The fluorosis problem in livestock production. Pub. 824.

————: 1960b. Nutrient requirements for poultry. Pub. 827.

Norris, L. C., Heuser, G. F., Ringrose, A. T., and Wilgus, H. S., Jr.: 1934. Studies of the calcium requirement of laying hens. Poultry Sci 13:308; also unpublished results Cornell Univ. Agr. Exper. Sta.

————, Heuser, G. F., Wilgus, H. S., Jr., and Ringrose, A. T.: 1933. The calcium and phosphorus requirements of laying hens. Cornell 46th Annual Rep., p. 137.

————, Leach, R. M., Jr., and Zeigler, T. R.: 1957. Requirements of poultry for molybdenum, zinc and other trace elements. Proc. Cornell Nutr. Conf for Feed Manufacturers, p. 33.

Nugara, D., and Edwards, H. M., Jr.: 1961. The effect of calcium and phosphorus on the magnesium requirement of chicks. Poultry Sci. 40:1438.

O'Dell, B. L., and Savage, J. E.: 1957a. Potassium, zinc and distillers dried solubles as supplements to a purified diet. Poultry Sci. 36:459.

————, and Savage, J. E.: 1957b. Symptoms of zinc deficiency in the chick. Federation Proc. 16:394.

Panda, J. N., and Combs, G. F.: 1950. Studies on the energy requirement of the chick for rapid growth. Poultry Sci. 29:774.

Patterson, E. L., Milstrey, R., and Stokstad, E. L. R.: 1957. Effect of selenium in preventing exudative diathesis in chicks. Proc. Soc. Exper. Biol. and Med 95:617.

Patterson, F. D.: 1928. Gout in poultry. Vet. Med. 23:73.

Patton, A. R.: 1939. A study of glycine toxicity. Poultry Sci. 18:31.

———, Hill, E. G., and Foreman, E. M.: 1948a. Amino acid impairment in casein heated with glucose. Science 107.623.

———, Hill, E. G., and Foreman, E. M.: 1948b. The effect of browning on the essential amino acid content of soy globulin. Science 108.659.

Wilgus, H. S., Jr., and Harshfield, G. S.: 1939. The production of goiter in chickens. Science 89:62.

Poley, W. E., and Moxon, A. L.: 1938. Tolerance levels of seleniferous grains in laying rations. Poultry Sci. 17:72.

———, Wilson, W. O., Moxon, A. L., and Taylor, J. B.: 1941. The effect of selenized grains on the rate of growth in chicks. Poultry Sci. 20:171.

Potter, L. M., and Matterson, L. D.: 1960. Metabolizable energy of feed ingredients for the growing chick. Poultry Sci. 39.781.

———, Matterson, L. D., Arnold, A. W., Pudelkiewicz, W. J., and Singsen, E. P.: 1960. Studies in evaluating energy content of feeds for the chick. I. The evaluation of the metabolizable and productive energy of alpha cellulose. Poultry Sci. 39:1166.

Prentice, J. H.: 1933a. The role of salt in poultry nutrition. I. Salt in the nutrition of the chick. Jour. Ministry Agr. Northern Ireland 4:72.

———: 1933b. The role of salt in poultry nutrition. II. Salt in the nutrition of the laying hen. Jour. Ministry Agr. Northern Ireland 4:92.

Quigley, G. D., and Waite, R. H.: 1932. Salt tolerance of baby chicks. Md. Agr. Exper. Sta., Bul. 340:343.

Reid, B. L., Kurnick, A. A., Svacha, R. L., and Couch, J. R.: 1956. The effect of molybdenum on chick and poult growth. Proc. Soc. Exper. Biol. and Med. 93.245.

Reiser, R.: 1950. The metabolism of polyunsaturated fatty acids in growing chicks. Jour. Nutr. 42:325.

Riesen, W. H., Clandinin, D. R., Elvehjem, C. A., and Cravens, W. W.: 1947. Liberation of essential amino acids from raw, properly heated, and overheated soybean oil meal. Jour. Biol. Chem. 167:143.

Roberts, R. E.: 1937. Salt tolerance of turkeys. Poultry Sci. 36.672.

Robertson, E. I., Miller, R. F., and Heuser, G. F.: 1948. The relation of energy to fiber in chick rations. Poultry Sci. 27:736.

Rogler, J. C., Parker, H. E., Andrews, F. N., and Carrick, C. W.: 1961. The iodine requirements of the breeding hen. II. Hens reared on a diet deficient in iodine. Poultry Sci. 40:1554.

Russell, W. C., Taylor, M. W., and Polskin, L. J.: 1940. Fat requirements of the growing chick Jour. Nutr. 19:555.

———, Taylor, M. W., Walker, H. A., and Polskin, L. J.: 1942. The absorption and retention of carotene and vitamin A by hens on normal and low fat rations. Jour. Nutr. 24:199.

Schaible, P. J., Bandemer, S. L., and Davidson, J. A.: 1938. The manganese content of feedstuffs and its relation to poultry nutrition. Mich. Agr. Exper. Sta., Tech. Bul. 159.

———, Moore, J. M., and Conolly, R. A.: 1933. Factors influencing the incidence of perosis in Barred Rock chicks. Poultry Sci. 12:524.

Schlotthauer, C. F., and Bollman, J. L.: 1934. Experimental gout in turkeys. Proc. Staff Meetings Mayo Clin. 9:560.

Schweigert, B. S., German, H. L., and Garber, M. J.: 1948. Synthesis of nicotinic acid from tryptophan by the developing chick embryo. Jour. Biol. Chem. 174:383.

Scott, H. M., Matterson, L. D., and Singsen, E. P.: 1947. Nutritional factors influencing growth and efficiency of feed utilization. The effect of the source of carbohydrate. Poultry Sci. 26:554.

———, Moeller, M. W., and Hinners, S. W.: 1956. Studies on purified diets. 1. Supplemental magnesium levels. Poultry Sci. 35:1169.

Scott, M. L., and Heuser, G. F.: 1951. Studies in duck nutrition. II. Studies of protein and unidentified vitamin requirements. Poultry Sci. 30:164.

———, Hill, F. W., Parsons, E. H. Jr., Bruckner, J. H., and Dougherty, E. III.: 1959. Studies on duck nutrition. 7. Effect of dietary energy: Protein relationships upon growth, feed utilization and carcass composition in market ducklings. Poultry Sci. 38.497.

———, Heuser, G. F., and Norris, L. C.: 1948. Energy, protein and unidentified vitamins in poult nutrition. Poultry Sci. 27:773.

———, Bieri, J. G., Briggs, G. M., and Schwarz, K.: 1957. Prevention of exudative diathesis by factor 3 in chicks on vitamin E-deficient Torula yeast diets. Poultry Sci. 36:1155.

Shutze, J. V., and Jensen, L. S.: 1963. Influence of linoleic acid on egg weight. Poultry Sci. 42.921.

Sibbald, I. R., and Slinger, S. J.: 1962. The metabolizable energy of materials fed to growing chicks. Poultry Sci. 41:1612.

Singsen, E. P.: 1947. Nutritional factors influencing growth and efficiency of feed utilization. II. The effect of protein level. Poultry Sci. 26:555.

———, Matterson, L. D., and Scott, H. M.: 1947. Phosphorus in poultry nutrition. III The relationship between the source of vitamin D and the utilization of cereal phosphorus by the poult. Jour. Nutr. 33:13.

———, Spandorf, A. H., Matterson, L. D., Serafin, J. A., and Tlustohowicz, J. J.: 1962. Phosphorus in the nutrition of the adult hen. I. Minimum phosphorus requirements. Poultry Sci. 41:1401.

Spillema, B.: 1936. Studies on the sodium requirement of chickens and on the consequences of a diet almost free of sodium. Tierernährung 7:184.

Slinger, S. J., Pepper, W. F., and Motzok, I.: 1950. Factors affecting the salt requirements of chickens. Poultry Sci. 29:780.

Smith, B. L.: 1959. Studies in the stability of vitamins A and D. II. Action of fatty peroxides on vitamin A. Biochem. Jour. 53.201.

Smith, E. V., and Weaver, J. E.: 1956. Turkeys. Cornell Ext. Bul. 559.

Snetsinger, D. C., Waibel, P. E., and Fitzsimmons, R. C.: 1962. Studies with crystalline amino acid diets for the turkey poult. Poultry Sci. 41:1428.

Stutz, M. W., and Matterson, L. D.: 1962. Metabolizable energy of animal by-products for the growing chick. Poultry Sci. 41:1617.

Suffran, F.: 1909. Poisoning of poultry by salt (Trans. title). Rev. Gen. de méd. vét. 13.698.

Sullivan, T. W.: 1962. The magnesium requirement of turkeys. 0–4 weeks of age. Poultry Sci. 41:1666.

Sunde, M. L.: 1956. A relationship between protein level and energy level in chick rations. Poultry Sci. 33:550.

Supplee, W. C.: 1955. A study of the effect of the significant variations of the calcium content of the A.O.A.C. basal rachitic ration on the percentage of bone ash in chick tibiae. Jour, Assn. Official Agr. Chem 18:146.

———, and Combs, G. F.: 1960. The effect of a dietary antibiotic on the requirement of the turkey poult for potassium. Poultry Sci. 39.1211.

Tepper, A. E., Durgin, R. C., and Charles, T. B.: 1939. Protein requirements of chickens at various stages of growth and development. N.H. Agr. Expct. Sta., Bul. 312.

Titus, H. W., Byerly, T. G, Ellis, N. R., and Nestler, R. B.: 1937. Effect of the calcium and phosphorus content of the diet of chickens on egg production and hatchability. Poultry Sci. 16:118.

Tomhave, A.: 1939. Protein levels of rations for White Leghorn pullets. Del. Agr. Exper. Sta., Bul. 219.

Torrey, J. P., and Graham, R.: 1935. A note on experimental salt poisoning in ducks. Cornell Vet. 25:50

Turpenen, O.: 1938. Further studies on the unsaturated fatty acids essential in nutrition. Jour. Nutr 15:351.

Watkins, W. E., and Mitchell, H. H.: 1936 The phosphorus requirements of growing chickens, with a demonstration of the value of controlled experimental feeding. Poultry Sci. 15:32.

Welch, H.: 1928. Goiter in farm animals. Mont. Agr. Exper. Sta., Bul. 214.

Wheeler, R. S., and James, E. C., Jr.: 1950. The problem of wet poultry house litter: Influence of total dietary protein and soybean meal content on water intake and urinary and fecal water elimination in growing chickens. Poultry Sci. 29.496.

Wiese, A. C., Benham, G. H., Elvehjem, C. A., and Hart, E. B.: 1941. Further bone phosphatase studies in chick perosis. Poultry Sci. 20.255.

———, Johnson, B. C., Elvehjem, C. A., Hart, E. B., and Halpin, J. G.: 1939. A study of blood and bone phosphatase in chick perosis. Jour. Biol. Chem. 127:411.

Wilcox, R. A., Carlson, C. W, Kohlmeyer, W., and Gastler, G. F.: 1955. The availability of phosphorus from different sources for poults fed practical type diets. Poultry Sci. 34:1017.

Wilgus, H. S., Jr.: 1931. The quantitative requirements of the growing chick for calcium and phosphorus. Poultry Sci. 10:107.

———, Gassner, F. X., Patton, A. R., and Gustavson, R. G.: 1941a. The goitrogenicity of soy-beans. Jour. Nutr. 22:43.

———, Harshfield, G. S., Patton, A. R., Ferris, L. P., and Gassner, F. X.: 1941b. The iodine requirements of growing chickens Poultry Sci. 20.477.

———, Norris, L. C. and Heuser, G. F.: 1936. The role of certain inorganic elements in the cause and prevention of perosis. Science 84,252.

———, Norris, L. C., and Heuser, G. F.: 1937a. The role of manganese and certain other trace elements in the prevention of perosis. Jour. Nutr. 14.155.

———, Norris, L. C., and Heuser, G. F.: 1937b. The effect of various calcium and phosphorus salts on the severity of perosis Poultry Sci. 16:232.

Winter, A. R., Dakan, E. L., and Bayes, A.: 1932. Protein levels for finishing pullets. Poultry Sci. 11:30.

Zeigler, T. R., Leach, R. M., Jr., and Norris, L. C.: 1958. Studies on the zinc requirement of the chick. Federation Proc. 16:498.

———, Leach, R. M., Jr., Norris, L. C., and Scott, M. L.: 1961. Zinc requirement of the chick: factors affecting requirement. Poultry Sci. 40.1584

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7

Vitamins and Vitamin Deficiencies

The term "vitamin" represents a heterogeneous group of fat-soluble and water-soluble chemical compounds, essential in nutrition, which bear no structural or necessary functional relationship to each other. All recognized vitamins, with the exception of vitamin C, are dietary essentials for poultry. Although the amounts of the various vitamins needed in poultry diets range from parts per million down to parts per billion, each of them is required in very exacting amounts for normal metabolism and health. Many of them function as integral parts of vital enzymes. All appear to play various catalytic roles in the many chemical reactions which are concerned in digestion, intermediary metabolism, anabolism, and catabolism within the animal body.

A marked deficiency of a single vitamin in the diet of the chick or turkey poult results in breakdown of the metabolic process in which that particular vitamin is concerned. This causes a vitamin deficiency disease which in some instances exhibits characteristic gross or histopathologic changes. In several instances a disease may occur as a result of a deficiency of any one of several vitamins. Perosis, for example, occurs in young chicks or poults when the diet is deficient in manganese or in any one of the following vitamins: choline, niacin, biotin, or folic acid. Furthermore, the symptoms and pathological changes of several different vitamin deficiencies are very similar in appearance. For example, the gross changes of either pantothenic acid or biotin deficiencies appear as severe dermatoses of the feet and of the areas around the mandibles and the eyes. In this instance, examination of the diet might be necessary to determine the cause of the deficiency.

Ataxia, with characteristic head retractions and convulsive movements, has long been associated with the vitamin E deficiency disease of chicks known as encephalomalacia. Similar symptoms also occur as a result of a severe deficiency of vitamin A in young chicks. In this case histological examination of the brains of chicks suffering from these deficiencies shows that encephalomalacia is caused by a hemorrhagic degeneration in the cerebellum

TABLE 7.1
THE VITAMIN REQUIREMENTS OF POULTRY
(Approximate amounts required per pound of diet)

	Chickens				Turkeys			Ducks
	0–8 wks.	8–18 wks.	Laying hens	Breeding hens	0–8 wks.	8–16 wks.	Breeding turkeys	0–8 wks
Vitamin A, I.U.	1,200	1,200	2,000	2,000	2,400	2,400	2,400	2,400
Vitamin D₃, I.U.	90	90	225	225	400	400	400	100
Vitamin E, I.U.	7	?	?	10	10	8	15	?
Vitamin K, mg.	0.4	0.4	0.4	0 4	0 8	0 8	0.8	0 8
Thiamine (vit. B₁), mg.	0.8	0.8	9 8	1 0	0 9	0 8	1.0	1.0
Riboflavin (vit. B₂), mg.	1.3	0.8	1.0	1.7	1 7	1 5	1.5	1 8
Pantothenic acid, mg.	4.2	4.2	2.1	4 2	5 0	4 5	8 0	5.0
Niacin, mg.	12	10	10	12	35	30	15	25
Pyridoxine (vit. B₆), mg.	1 3	?	1.3	2 0	2 0	1.6	2 0	1.2
Biotin, mg.	0 04	?	?	?	0.06	0.05	0.05	0 05
Folic acid (Folacin), mg.	0 25	?	0.11	0 16	0 4	0 4	0 78	0.4
Choline, mg.	600	450	450	450	900	800	650	650
Vitamin B₁₂, mg.	0 004	?	?	0 002	0 005	0.003	0.005	?

which is not evident in chicks suffering from the ataxia of vitamin A deficiency.

Usually, after diagnosis of a specific vitamin deficiency, confirmatory evidence can be obtained upon determining, by calculation or analysis, the vitamin content of the ration that was fed to the chicken or turkey and comparing the level found with the known requirement for that particular vitamin.

Therefore, anyone interested in vitamin deficiency diagnosis in poultry must not only be able to recognize the symptoms and lesions of vitamin deficiency diseases, but also must know the vitamin requirements of the various classes of poultry at different stages of development, the vitamin content of the ingredients commonly used in poultry rations, and the metabolic dysfunctions which may result from a deficiency of each of the known vitamins.

Vitamin requirements of poultry. The minimum vitamin requirements of chickens, turkeys, and ducks are presented in Table 7.1. These values were taken in part from the report of the Committee on Animal Nutrition of the National Academy of Sciences — National Research Council (NAS-NRC) on the "Nutrient Requirements for Poultry" (1960).

While all of the vitamins listed in Table 7.1 are needed by poultry, only those require special attention in the formulation of poultry rations which are not present in adequate amounts in the cereal grains and protein supplements used in the rations.

Vitamin A, vitamin D, and riboflavin are the vitamins most apt to be deficient if special attention is not given to provide them when the feed is formulated. Recently, however, due to continued extraction and purification of many common ingredients, and due to the tendency to omit animal proteins and high-fiber ingredients such as alfalfa meal and wheat mill by-products, the amounts of several of the other vitamins have decreased to levels which are sometimes deficient. These are vitamin E, vitamin K, pantothenic acid, vitamin B₁₂, niacin, and choline.

Vitamin content of poultry feed ingredients. The vitamin contents of the common poultry feed ingredients are presented

TABLE 7.2

THE VITAMIN CONTENT OF POULTRY FEEDSTUFFS

Feed	Riboflavin	Niacin	Pantothenic Acid	Choline	Vitamin B₁₂	Vitamin A
	(mg/lb)	(mg/lb)	(mg/lb)	(gm/lb)	(µg/lb)	(I.U./lb)
Barley	.8	24	3.7	.53		2,000
Corn, yellow	.5	10	2.6	.20		5,000
Hominy feed, yellow . .	1 1	20	3 9	.44		
Milo	.4	13	5 0	.43		
Oats	.4	6	6 8	.43		
Wheat, soft	.5	27	5 2	.45		
Wheat, hard	.5	27	8 0	.45		
Flour middlings . . .	.9	43	9 0	.49		
Standard middlings . .	.9	45				
Soybean oil meal, 50% .	1 5	12	6 6	1 25		
Soybean meal, 44% . .	1.5	12	6 6	1 25		12,000
Corn gluten meal . .	.7	23	4 7	.15		
Fish meal, menhaden .	2 2	25	4 0	1.60	40	
Fish solubles	6 0	120	17	1 40	100	
Meat scraps, 55% . .	2 4	26	2 2	.90	20	
Meat and bone scrap, 50% .	2 0	22	1 7	1 00	20	
Liver and gland meal .	18	73	48	4 80	230	
Brewers dried yeast	16	200	50	1 80		
Corn distillers solubles .	7 7	52	9 5	2 20	1	500
Skim milk, dried . .	9 1	5	15	.65	20	
Whey, dried	14		22	.90	7	
				45		105,000
Alfalfa leaf meal .	7 4	17	19	40		70,000
Alfalfa meal	7 3	9	12			
Vitamin feed oils .						1,000,000 to 2,000,000
Stabilized vitamin A supplemens . .						2,000,000 to 150,000,000

in Table 7.2. Values are presented for all of the vitamins that are presently considered to be important in the formulation of poultry rations.

From the data in this table it is possible to calculate the vitamin content of most poultry rations when these rations are made up of ingredients of average quality. Poor quality ingredients, of course, cannot be expected to supply the amounts of the vitamins listed in the table.

Present-day poultry rations are usually formulated to contain more than adequate amounts of all of the vitamins listed in Table 7.1 in order to provide margins of safety to compensate for possible losses during feed processing, transportation, storage, and for variations in feed composition and environmental conditions.

If a deficiency should occur, it is usually due either to the inadvertent omission of a critical ingredient during mixing of the feed or to the destruction of one or more of the vitamins during processing of that ingredient. The vitamins which are most prone to suffer destruction are the fat-soluble vitamins, A, D, and E. Under very severe conditions of processing or storage, thiamine and/or pantothenic acid may be destroyed. The special considerations in regard to each vitamin will follow.

VITAMIN A AND VITAMIN A DEFICIENCY

Vitamin A is essential in poultry rations, not only for growth but also for optimum vision and for maintaining the integrity of the mucous membrane. Since this mem-

brane composes the epithelium lining all of those canals and cavities of the body which communicate with the external air, such as the alimentary canal and its branches, the respiratory tract and its connections, and the genito-urinary tract, these are the areas in which lesions of vitamin A deficiency may be detected, either grossly or histologically, depending upon the degree of the deficiency.

Chemical nature of vitamin A. Vitamin A is a fat-soluble, unsaturated, primary alcohol containing one beta ionone ring and a long side chain composed of a series of isoprene units containing conjugated double bonds. The empirical formula is $C_{20}H_{30}OH$. Because of its conjugated system of double bonds, vitamin A is very easily oxidized unless precautions are taken to prevent its destruction. Quackenbush et al. (1942) and others have shown that vitamin E and other natural antioxidants in feeds are essential for the protection of vitamin A. On the other hand, Sumner and Dounce (1939) point out that certain legumes, particularly soybeans, contain an enzyme known as carotene oxidase, which readily destroys the carotenes and xanthophylls, and probably also destroys vitamin A. Proper heat treatment of soybeans, however, should destroy this enzyme.

In view of the difficulty in being assured of adequate vitamin A in poultry and livestock feeds, modern producers of vitamin A supplements have undertaken a great deal of research on methods of stabilizing this vitamin. They have succeeded in greatly enhancing the stability of vitamin A in two ways: (1) by mechanical means, wherein minute droplets of vitamin A are enveloped in a stable fat, gelatin, or wax, forming a small bead which prevents most of the vitamin A from coming into contact with oxygen until it is digested in the intestinal tract of the animal; and (2) through the use of effective antioxidants which markedly prolong the induction period which precedes active oxidation of vitamin A, thereby allowing the vitamin A to be consumed by the

animals before this oxidation takes place. The chief antioxidant in current use is 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin).

Occurrence. Vitamin A is found only in animal tissue, where it is stored largely in the liver. Although cod liver oil is the most noted source of vitamin A, the livers of shark, tuna, halibut, and many other salt-water species are rich sources of this vitamin. It occurs both as the free alcohol and esterified with various organic acids. Vitamin A, usually as acetate and palmitate esters, is also produced synthetically in this country on a commercial scale. The livers of fresh-water fish contain a biologically active derivative of vitamin A which is known as vitamin A_2. Synthetic and natural vitamin A_2 possess approximately 30 to 40 per cent of the biological activity of vitamin A for poultry.

Pro-vitamins A. Since animals do not have the ability to synthesize vitamin A, all of the natural vitamin A found in body tissues arises originally from precursors of vitamin A which are synthesized by green plants and stored in the green leaves, fruit, and yellow seeds of these plants. These precursors are known as carotenoids. All carotenoids which are precursors of vitamin A contain within their structure at least one complete vitamin A unit with the exception of the alcohol functional group which is attached to the vitamin A moiety by the animal during absorption and degradation of the carotenoid in the intestinal wall of the chick, poult, or other animal. The most abundant precursor of vitamin A in nature is beta-carotene which contains two vitamin A moieties attached to each other. The evidence on the metabolism of beta-carotene indicates, however, that one end of the beta-carotene molecule is destroyed in its conversion to vitamin A, thus allowing only one molecule of vitamin A from each molecule of beta-carotene (Glover and Redfearn, 1954). Beta-carotene is the predominant carotenoid found in green leaves of legumes such as alfalfa

clover and also in green grasses and green vegetables. The other vitamin A precursor of importance in poultry nutrition is cryptoxanthin which is the carotenoid present in yellow corn.

The xanthophylls. In addition to the vitamin A precursors present in green and yellow plants, these materials also contain a number of vitamin A inactive chemical substances closely related to the vitamin A precursors. These substances are known collectively as the xanthophylls. Xanthophylls are absorbed by poultry and are deposited in the eyes, beaks, shanks, adipose tissue, and in the egg yolks. The vitamin A inactive xanthophylls, mainly lutein and zeaxanthin, together with the vitamin A precursor, cryptoxanthin, are largely responsible for the pigmentation of chickens, the color in the iris of the eye, and the yellow color of the yolks. Ganguly *et al.* (1953) showed that beta-carotene is not deposited as such in the chicken or in eggs. Although lutein and zeaxanthin have no vitamin A activity, these xanthophylls may act, to some extent, as antioxidants, and thereby help to preserve vitamin A and its precursors.

Vitamin A requirements of poultry. The vitamin A requirements of poultry of the NAS-NRC (1960) are given in Table 7.1. In arriving at these requirements, the Subcommittee on Poultry Nutrition of the NAS-NRC Committee on Animal Nutrition has considered over 25 reports of research work on the vitamin A requirements of poultry. A review of these reports shows that the vitamin A requirements listed in Table 7.1 were determined with the use of fish liver oils, especially "Reference" cod liver oil, as the sources of vitamin A. In recent research by Hill *et al.* (1961) it was shown that when present-day, commercially stabilized vitamin A preparations are used, the vitamin A requirement of laying and breeding hens approximates 1,200–1,600 I.U. of vitamin A per pound of ration, and the vitamin A requirement of starting chicks is no higher than 600 I.U. per pound of ration. These

results indicate that the true vitamin A requirements of all poultry are considerably less than previously believed, and that the higher apparent requirements are due to oxidative destruction of a large portion of the vitamin A when unstabilized fish oils are used in the course of determining the vitamin A requirements.

Symptoms

Adult chickens. When adult chickens are placed on a diet severely deficient in vitamin A, symptoms develop usually within 2 to 5 months, the length of time depending upon the amount of vitamin A stored in the liver and other tissues of the body. As the vitamin A deficiency progresses, the chickens become emaciated and weak and their feathers are ruffled (Beach, 1924). According to Sherwood and Fraps (1932), there is a marked decrease in egg production, and the length of time between clutches increases greatly. Polk and Sipe (1940) reported that a deficiency of vitamin A causes a great deficiency in hatchability and an increase in embryonic malpositions and mortality in eggs from affected birds. A watery discharge from the nostrils and eyes is noted and the eyelids are often stuck together (Beach, 1924). As the deficiency continues, an accumulation of milky white, caseous material forms in the eyes. In this stage of the disease, the eyes become filled with this white exudate to such an extent that it is impossible for the chicken to see unless the mass is removed; in many cases the eye is destroyed (Beach, 1924; Sherwood, 1939).

Chicks. When day-old chicks are given a vitamin A-deficient diet, the first symptoms may appear at the end of the first week if the chicks were progeny of hens receiving a diet low in vitamin A. On the other hand, if the chicks were progeny of hens receiving adequate amounts of vitamin A, symptoms and lesions of vitamin A deficiency may not appear until the chicks are six or seven weeks of age, even though they are receiving a diet completely

devoid of vitamin A. This is due to the fact that hens receiving adequate amounts of vitamin A store large quantities in their eggs which consequently provide the day-old chick with sufficient reserves to last for an extended period.

Vitamin A deficiency symptoms in chicks are characterized by a cessation of growth, by drowsiness, weakness, incoordination, emaciation, and ruffled plumage. If the deficiency is severe, the chicks show an ataxia not unlike the ataxia of vitamin E deficiency known as encephalomalacia or crazy chick disease (Adamstone, 1947; Hill et al., 1961). A better understanding of the cause of ataxia in vitamin A-deficient chicks may arise from the discovery by Woollam and Millen (1955) that one of the earliest signs of deficiency is an increased pressure of the cerebrospinal fluid. The yellow pigment in the shanks and beaks in breeds of chickens that usually contain this pigment is lost, and the combs and wattles of the chicks are usually pale. In acute vitamin A deficiency, lacrimation may occur and a cheesylike material is seen under the eyelids. Xerophthalmia is a definite symptom of vitamin A deficiency, but all chicks do not exhibit this symptom, because in the acute deficiency they often die of other causes before the eyes become affected.

Turkeys. The vitamin A requirements of turkeys are given in Table 7.1. The length of time required to produce vitamin A deficiency in poults is similar to that required for chicks and depends upon the amount of vitamin A carried over from the hen through the egg to the poult at hatching. Usually vitamin A deficiency symptoms appear in about 4 weeks. If continued on a vitamin A-deficient diet, the turkeys will all die in about 6 weeks (Hinshaw and Lloyd, 1934; Stoewsand and Scott, 1961). Symptoms and lesions of vitamin A deficiency in the turkey are described in Chapter 41.

Vitamin A and bone development. According to Wolbach and Hegsted (1952, 1953), vitamin A deficiency in young ducks

causes marked retardation and suppression of endochondral bone growth, and excess vitamin A produces an acceleration of this bone development. This may be due to alterations in the alkaline phosphatase content of the epiphyseal junction of the bone in ducks similar to that which was shown by Ludwig (1955) for vitamin A deficiency and hypervitaminosis A in rats. The studies reviewed by Wolf and Johnson (1960), however, indicate that effects of vitamin A upon bone development may be secondary to its effects upon mucopolysaccharide biosynthesis in cartilage and connective tissues.

Vitamin A and internal egg quality. Bearse et al. (1953a) reported that the incidence and severity of blood spots in eggs of two different strains of White Leghorn chickens was progressively increased as the level of vitamin A in the diet of the hens was decreased. Recent experiments conducted at Cornell University (Hill et al., 1961) confirm the finding that blood spots are increased in number and severity when hens are fed vitamin A deficient diets. However, these studies showed that the amount of vitamin A required to minimize blood spot incidence is not higher than the vitamin A requirement for good production and health of the laying hens. Certain strains of chickens show a fairly high incidence of blood spots in their eggs which cannot be improved by increasing the vitamin A level in the diet above that needed for satisfactory egg production and health.

Vitamin A, intestinal coccidiosis, and other intestinal parasites. Davies (1952) found that in chickens receiving adequate vitamin A in the form of natural sources of beta-carotene and cryptoxanthin, infecting one group with intestinal coccidiosis caused a reduction in liver stores of vitamin A such that the infected birds had less than 10 per cent as much liver vitamin A as the uninfected controls. These results were confirmed by Erasmus, Scott, and Levine (1960), who showed that although the severity of experimen-

'ly-induced coccidiosis was similar in :'·-ks receiving minimum requirements of vitamin A as compared to chicks receiving higher levels of the vitamin, recovery of surviving chicks, as measured by improved appetites and growth rates, was enhanced as the level of vitamin A in the diet was increased up to a level equivalent to ten times the minimum requirement under normal, nonstress conditions.

Ackert and associates (1931) reported that vitamin A-deficient chicks showed significantly larger numbers and longer intestinal roundworms (*Ascaridia lineata*) than were found in comparable chicks receiving adequate vitamin A. This work has been criticized on the basis that it is very difficult to determine total numbers of roundworms in the intestinal tract of chickens and that the number may vary considerably from day to day.

Pathology

Adult chickens. According to Seifried (1930a), when adult chickens are placed on a diet lacking vitamin A, lesions first appear in the upper alimentary tract and are largely confined to the mucous glands and their ducts. The original epithelium becomes replaced by a stratified squamous,

keratinizing epithelium which blocks the ducts of the mucous glands causing them to become distended with secretions and necrotic materials. Small, white pustules are found in the nasal passages, mouth, esophagus, pharynx, and may extend into the crop. The pustules may range in size from microscopic lesions to 2 mm. in diameter (Fig. 7.1). As the vitamin A deficiency progresses, the lesions become larger and are raised above the surface of the mucous membrane and show a depression in the center. Small ulcers surrounded by inflammatory products may appear at the site of these lesions. This condition closely resembles certain stages of fowl pox, and the two conditions can be differentiated only by microscopic examination. Due to the breakdown of the original mucous membrane, bacteria, viruses, and other pathogenic microorganisms may invade these tissues and enter the body, thereby producing infections which are secondary to the original vitamin A deficiency symptoms.

Chicks. Young chicks suffering from chronic vitamin A deficiency show lesions in the mucous membranes of the head, esophagus, crop, and respiratory tract. The kidneys become pale and show a network
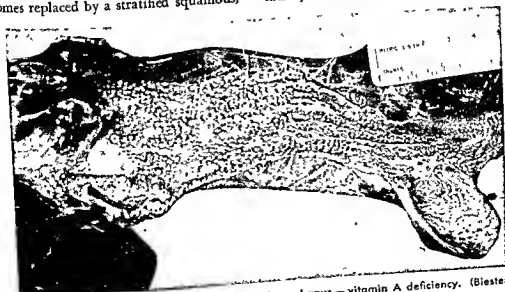
FIG. 7.1 – Pustulelike lesions in pharynx and esophagus — vitamin A deficiency. (Biester and Schwarte, No. Am. Vet.)

of fine white lines. These are the renal tubules filled with white urates. In extreme cases, even the ureters are filled with urates. According to Elvehjem and Neu (1932), the blood level of uric acid increases from a normal of about 5 mg. to as high as 44 mg. per 100 cc. of whole blood during severe vitamin A deficiency. Deposits of urates have been found on the heart, pericardium, liver, and spleen of affected birds. Elvehjem and Neu found that vitamin A deficiency does not disturb uric acid metabolism, but injures the kidney in such a way as to prevent normal excretion of uric acid.

The clinical symptoms and pathological lesions of vitamin A deficiency of the respiratory tract are variable, and it is difficult to differentiate this condition from infectious coryza, virus diphtheria, and infectious tracheal bronchitis (Beach, 1924; Seifried, 1930b). In vitamin A deficiency, thin membranes and nasal plugs appear but are usually limited to the cleft palate and its adjacent epithelium. They may be removed easily without bleeding. According to Seifried, this is not true in virus diphtheria. Atrophy and degeneration of the respiratory mucous membrane and its glands occur. Later the original epithelium is replaced by a stratified squamous, keratinizing epithelium. In the early stages of vitamin A deficiency in chickens, the turbinates are filled with seromucoid waterclear masses, which may be forced out of the nodules and cleft palate by the application of slight pressure. In the early stage of the disease, the vestibule becomes plugged and overflows into the paranasal sinuses. The exudate may also be forced through the cleft palate producing a white, or slightly yellow, caseous mass which fills the sinuses and other nasal cavities. This causes a swelling of one or both sides of the face. After the sinuses have filled, the tear ducts become occluded. The eyeball is pressed against the frontal bones and is sometimes forced out to the side because it cannot move in a ventral direction. Upon removal of the inflammatory products, the mucous membranes of the nasal passages, sinuses, mouth, and throat appear thin, rough, and dry. Unattached masses of caseous material often form in the cleft palate and in the mucous membranes of the roof of the mouth.

Lesions in the larynx and trachea of vitamin A-deficient chickens occur both in the early and in the later stages of the disease. Near the entrance of the pharynx, the lesions consist of pustulelike patches of white, caseous material. Caseous and crumbly white masses often appear in the mucous membrane on the ventral side of the anterior end of the larynx, and in the pointed angle which is formed by the cartilage of the larynx. Frequently, similar lesions may be found in the trachea and bronchi. In the early stages these may be difficult to see. As the condition progresses, the mucous membrane is covered with a dry, dull, and fine film, which is slightly uneven, whereas the normal membrane is even and moist. In some cases, small, nodulelike particles are in or beneath the mucous membrane in the upper part of the trachea. These lesions are much more striking in the latter stages of the deficiency and may then be seen easily with the naked eye. The formation of a thin membranous covering over the mucosa of the trachea and bronchi is a symptom of vitamin A deficiency, but is often mistaken for the symptom of infectious tracheitis (Fig. 7.2). The smaller bronchi often become completely occluded by these membranes. As a result, in some cases the most marked changes appear in the larynx, while in others these appear in the trachea.

Histopathology

The first histologic lesion of vitamin A deficiency is an atrophy of the cytoplasm and a loss of the cilia in the columnar ciliated epithelium (Seifried, 1930a). The nuclei often present marked karyorrhexis. A pseudomembrane, formed by the atrophying and degenerating ciliated cells, may hang as tufts on the basement membrane; later these are sloughed. During this process, new cylindrical or polygonal cells may be formed either singularly or in

FIG. 7.2 — Trachea of chicken with A avitaminosis showing desquamated epithelium partly in the form of a tube. Bird died after 87 days on experimental diet. (Seifried, Jour. Exper. Med.)

pairs, and appear as islands beneath the epithelium. These new cells become more and more numerous, and their nuclei become larger and contain less chromatin as they develop. The cell boundaries become less clearly defined; finally, the columnar ciliated epithelial lining of the trachea, bronchi, and submucous glands becomes transformed into a squamous, stratified, keratinizing epithelium (Fig. 7.3). Seifried (1930b) concluded that this process is not related to bacterial infection.

The nasal cavities and communicating sinuses show essentially the same epithelial lesions as the trachea. All parts of the nasal cavities are usually involved. There is an increased proliferation of the superficial epithelial cells in the gland-free part of the nasal vestibule. A true keratinization, similar to that found in the trachea, appears in

the mucous membrane of the roof of the nasal vestibule. Seifried (1930b) found that the epithelium of the submucous glands becomes involved somewhat later than the epithelium of the mucous membrane. Keratinization occurs in the excretory duct of the lateral nasal gland and in the nasolacrimal duct.

Jungherr (1943) stated that histopathological examination of tissues from the nasal passages of chicks serves as the most sensitive indicator of borderline deficiencies of vitamin A. Chicks receiving borderline deficient levels of vitamin A show lesions which resemble, in basic character, but not in severity, those described by Seifried (1930b) for a complete deficiency of vitamin A. The specific lesion in both cases consists of squamous metaplasia of the secretory and glandular

FIG. 7.3 — Cross section through trachea showing newly formed stratified epithelium. Several cells near surface showing "balloon" degeneration. ×990. (Seifried, Jour. Exper. Med.)

FIG. 7.4 — Cross section through base of tongue showing early keratinization and degeneration of the upper layers of the newly formed epithelium. X80. (Seifried, Jour. Exper. Med.)

epithelium with inflammatory or obstructive changes secondary in nature. The metaplastic changes are first noted, microscopically, near the mucocutaneous junction in the septum or the medial convexity of the anterior turbinate.

Further evidence that vitamin A is directly concerned with the differentiation of the mucous membrane was obtained by Fell and Mellanby (1953), who showed that explants of chick ectoderm, when grown in a tissue culture supplemented with a high level of vitamin A, failed to develop into typical keratinized epithelium Instead, the cells differentiated into mucus-secreting, often ciliated columnar epithelial cells, resembling those of the nasal mucosa. Kahn (1954) extended these findings by showing that added vitamin A prevents keratinization that otherwise occurs in explants of rat vaginal epithelium.

Lesions in the glands of the tongue, palate, and esophagus are very much the same as those found in the respiratory tract (Seifried, 1930a). The early lesions occur in the collecting spaces and ducts and may appear first in the maxillary and submaxillary glands (Fig. 7.4). As the disease progresses, the collecting spaces become filled with masses of mucus, degenerated cells, and inflammatory products. The epithelium extends into the ducts, which become partially filled with the stratified, keratinized epithelium, and more or less complete occlusion results (Fig. 7.5). Desquamated cells from the newly formed stratified epithelium become more and more numerous. The glands become smooth and distended, although originally they were sacs with invaginations. These distended sacs finally become completely filled with stratified keratinized epithelial cells. Seifried believes that these lesions are responsible for the fact that bacterial infections are more prevalent in the mouth cavity than in the crop and esophagus during avitaminosis A.

Adamstone (1947) conducted investigations to differentiate, by histologic

citric acid metabolism, since Steenbock and Bellin (1953) and Bellin et al. (1954) showed that vitamin D increases the urinary excretion of citrate and the citrate content of bone, kidney, heart, and small intestine, but not of the livers of rats fed normal or rachitogenic diets. Steenbock and Herting (1955) concluded that vitamin D is required for growth and for normal functioning of citric acid metabolism, independently of its effects upon bone calcification.

Chemical nature and occurrence. Vitamin D is the general term applied to a number of fat-soluble sterol derivatives which are active in the prevention of rickets in animals. Although there are numerous chemically distinct forms of vitamin D, when measured by their capacity to prevent or cure rickets in rats or other mammals, only one form, activated 7-dehydro-cholesterol (vitamin D_3), has been found to have an appreciable effect in the prevention of rickets in poultry.

Vitamin D_3 is produced by irradiation of 7-dehydro-cholesterol with ultraviolet light, either from the sun or from an artificial source. It is synthesized by animals and is found in the skin, butter fat, egg yolk, fish oils, and other lipids throughout the animal body or its products. According to Koch and Koch (1941), the skin of the legs and feet of poultry contains about eight times as much provitamin D_3 as the body skin. Spectrophotometric examination of ether extracts from leg skin showed the presence of 7-dehydro-cholesterol in relatively high amounts. In contradiction of the claims of Hou (1928, 1931) the preen gland contained very little, if any, provitamin D_3. Upon being produced on the skin surface by irradiation, vitamin D_3 is absorbed through the skin and transported to the liver by the blood and stored there for future use. Thus tuna, halibut, and other liver oils are rich in vitamin D_3. However, in poultry rations the chief source of vitamin D_3 arises from the commercial separation and irradiation of animal sterols known as "D-activated animal sterols." From these, feed supplements of guaranteed potencies are prepared.

Vitamin D_2, which is effective for most mammals, is not a satisfactory antirachitic vitamin for poultry. Vitamin D_2 is produced by the irradiation of the plant sterol, ergosterol. Bethke et al. (1936) found that vitamin D from cod liver oil was approximately 10 times as active, on a rat unit basis, as irradiated ergosterol for egg production in hens maintained in strict confinement. Dihydrotachysterol has been shown by McChesney (1943), Fritz et al. (1945), and Motrok et al. (1946) to possess antirachitic activity for chicks. However, the activity of this compound is somewhat less than that of vitamin D_3. Cod liver oil and other fish liver oils appear to contain mixtures of antirachitic sterols. Therefore, these oils usually are not as active for poultry as vitamin D_3 (Singsen and Mitchell, 1945) when measured in equivalent doses of rat-active vitamin D. Boucher (1944) showed that in turkey poults, irradiated animal sterols and irradiated 7-dehydro-cholesterol were approximately twice as active as the vitamin D from reference cod liver oil or fortified sardine oil when fed on the basis of their A.O.A.C. unit potencies.

The International Unit of vitamin D is represented by an antirachitic activity for chicks equivalent to that of 0.025 microgram of crystalline vitamin D_3. In converting the older A.O.A.C. unit of vitamin D activity for chicks to International Units, the A.O.A.C. units must be reduced 25 per cent.

Vitamin D_3, like vitamins A and E, is unstable. Although Baird et al. (1939) detected no vitamin D destruction in a mixed feed stored at summer temperatures for 32 weeks, Norris et al. (1929) reported that the addition of cod liver oil to finely divided feed mixtures at the minimum protective dosage resulted in a material destruction of vitamin D, when the feed was stored at room temperature from 12 to 16 weeks preceding consumption.

a D-activated animal sterol supplement was premixed with aCl and $CaCO_3$ and stored for 3 weeks, of the vitamin D was destroyed (Milby and Thompson, 1943). Fritz et al. (1942) showed that vitamin D premixed with oystershell flour, dried whey, dried skim milk or mineral mixtures also lost most of its activity within one month. Miller et al. (1942) found that although 0.5 per cent $MnSO_4$ added to a premix of 9 per cent cod liver oil on bran caused destruction of both vitamin A and vitamin D, there was no destruction in a finished feed with or without the addition of 4 oz. of $MnSO_4$ per ton of feed. Although most mixed feeds apparently contain sufficient vitamin E and other natural antioxidants to prevent destruction of vitamin D, it now appears practicable to use small amounts of the synthetic antioxidant, ethoxyquin, to further protect the vitamin D potency of poultry feeds.

Vitamin D requirements of poultry. The vitamin D_3 requirements of poultry depend upon the sources of phosphorus in the ration, the amounts of and the ratio of calcium to phosphorus, and the extent of exposure to direct sunlight. Hart et al. (1923), Mussehl and Bancroft (1925), and Heuser and Norris (1929) showed that 11 to 45 minutes of sunshine, daily, was sufficient to prevent rickets in growing chicks, and that no further improvements in growth were obtained under these conditions by adding cod liver oil.

The vitamin D requirements of poultry, presented in Table 7.1, are sufficiently high to produce normal growth, calcification, production, and reproduction in the absence of sunlight, provided that the diets contain the recommended levels of calcium and available phosphorus. The requirements of turkeys for vitamin D are considerably higher than the requirements of chickens, although studies on the vitamin D requirements of turkeys have yielded conflicting results. Some studies have indicated turkey requirements to be even higher than those given in Table 7.1.

Further research is needed to determine the reason for the very high vitamin D requirements of turkeys as compared to chickens.

Symptoms

Mature chickens. According to Hughes and Payne (1924) and Doyle (1925), symptoms of vitamin D deficiency begin to occur in laying hens in confinement about 2 to 3 months after they are deprived of vitamin D. The first symptom is a marked increase in the numbers of thin-shelled and soft-shelled eggs, followed soon afterward by a marked decrease in egg production. Hatchability is also markedly reduced. This is due, according to Hart et al. (1925a), to a lack of calcium in the embryos from vitamin D-deficient hens.

Individual hens may show temporary loss of use of the legs, with recovery after laying an egg, usually a shell-less egg. During the periods of extreme leg weakness, the hens show a characteristic posture which has been described as a "penguin-type squat." Later, the beak, claws, and keel become very soft and pliable. The sternum usually is bent and the ribs lose their normal rigidity and turn inward at the junction of the sternal and vertebral portions. This produces a characteristic inward curve of the ribs along the sides of the thorax.

Chicks. In addition to retarded growth, the first symptom of vitamin D deficiency in chicks is rickets, which is characterized by a severe weakness of the legs. The beaks and claws become soft and pliable. This usually appears between 2 and 3 weeks of age. The chicks walk with obvious effort and take only a few unsteady steps before squatting on their hocks, whereupon they rest, but at the same time sway slightly from side to side, indicating a lack of complete sense of equilibrium. The feathering is poor and in New Hampshire (Glazener et al., 1946) and Buff Orpington × Rhode Island Red (Lillie and Bird, 1919) chicks, an abnormal blackening of the feathers is observed in vitamin D deficiency. Buff

Plymouth Rock chicks fed vitamin D-deficient rations, however, showed all of the symptoms of rickets, but did not show blackening of the feathers.

In chronic vitamin D deficiency, marked skeletal distortions become apparent. The spinal column may bend downward in the sacral and coccygeal region. The sternum usually shows both a lateral bend and an acute dent near the middle of the breast. These changes reduce the size of the thorax, with consequent crowding of the vital organs.

Turkeys. Vitamin D deficiency in turkeys is described in Chapter 41.

Pathology

Mature chickens. In hens receiving a deficient amount of vitamin D, the characteristic changes observed on postmortem are confined to the bones and parathyroid glands. The bones are soft and break easily. Well-defined knobs are present on the inner surface of the ribs where the sternal portions join the vertebral portions. Many of the ribs show evidence of spontaneous fracture in this region. Skeletal changes also appear in the vertebral column, pelvis, and sternum. Histological sections of the leg bones show a deficiency of calcium and an excess of osteoid tissue.

Chicks. The most characteristic internal signs of vitamin D deficiency in chicks are a beading of the ribs at their juncture with the spinal column and a bending of the ribs downward and posteriorly (Fig. 7.6).

Poor calcification can be observed at the epiphysis of the tibia or femur. By dipping the split bone in silver nitrate solution and allowing it to stand under an incandescent light for a few minutes, the calcified areas are easily distinguished from the areas of uncalcified cartilage (Fig. 7.7).

Hypervitaminosis D. Very high levels of vitamin D, 2,000,000 I.U. or more per pound of diet, cause renal damage. This is due to calcification of the kidney tubules, and usually calcification in the aorta and other arteries, especially the blood vessels of the spleen (Seifried and Heidegger,

FIG. 7.6 — Rickets in the chicken, showing severe beading and curvature of the ribs and spinal column.

1933; van Niekerk and Franken, 1937). Irradiated ergosterol (vitamin D_2) at high levels also is toxic to chickens (Hall and King, 1931; Bethke et al., 1936).

Frölich (1954) reported that vitamin D_3, at levels of 290 and 640 I.U. per pound of diet, inhibited growth in vitamin B_{12} deficient chicks. Addition of adequate vitamin B_{12} to the diet caused maximum growth at both levels of vitamin D, which was superior to that obtained with 90 I.U. of vitamin D_3 per pound of diet in the presence of adequate vitamin B_{12}.

Vitamin D and hormones. Vitamin D_3, at a level in the diet which was 10 times that required for prevention of rickets, caused increases in the linear measurements and weights of the combs, wattles, and gonads in New Hampshire cockerels at 14 and 18 weeks of age as compared to the measurements and weights of these tissues in cockerels of equal body weight receiving the minimum antirachitic dosage of vitamin D_3 (Buckner et al., 1951).

Landauer (1954) found that estrogen treatment of vitamin D-deficient cockerels
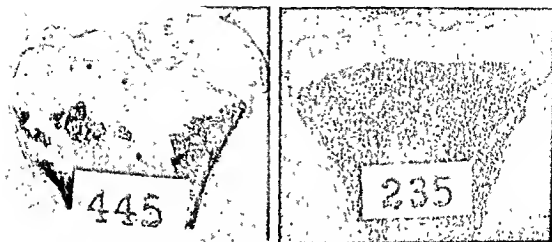
FIG. 7.7 — Tibia of a severely vitamin D-deficient, rachitic chick (445) and a normal chick (235), after staining with silver nitrate and exposure to light.

with daily intramuscular injections of 2 mg. of estradiol benzoate prevented loss of muscular control and reduced the severity of rickets, but had no effect upon the enlargement of the parathyroid glands during a 57-day experimental period.

McGinnis et al. (1947) found that the abnormal feather pigmentation in vitamin D-deficient New Hampshire chicks could be altered by thyro-active compounds and diethylstilbestrol. Iodinated casein tended to increase the amount of black pigment, while thiouracil or diethylstilbestrol decreased the deposition of black pigment. Thyroid hormone has been shown by Gutteridge and Novikoff (1947) also to be interrelated with vitamin D in the improvement of eggshell strength.

Treatment of vitamin D deficiency. Hooper et al. (1942) found that the feeding of a single massive dose of 15,000 I.U. of vitamin D_3 cured rachitic chicks more promptly than when generous levels of the vitamin were added to the feed. This single oral dose protected cockerels against rickets for a period of 8 weeks, and pullet chicks for 5 weeks. In giving massive doses to rachitic chicks, it should be remembered that excess vitamin D can be harmful. The dose should be scaled to the degree of the deficiency and the amount of vitamin D added to the feed should not be excessive.

VITAMIN E AND VITAMIN E DEFICIENCY

Vitamin E deficiency produces encephalomalacia, exudative diathesis, and muscular dystrophy in chicks, enlarged hocks, and dystrophy of the gizzard musculature in turkeys, and muscular dystrophy in ducks. It is also required for normal embryonic development in chickens, turkeys, and probably ducks. Prolonged vitamin E deficiency produces testicular degeneration and lack of fertility in male chickens.

Although the metabolic action of vitamin E is not known, Goldstein and Scott (1956) showed that the albumin level is markedly reduced in the plasma of vitamin E-deficient chicks. Creatine excretion is increased and muscle creatine levels are decreased in vitamin E-deficient chicks and poults.

In its alcoholic form, vitamin E is a very effective antioxidant. In this capacity, it is an important protector in feeds of the essential fatty acids and other highly unsaturated fatty acids as well as vitamin A, vitamin D_3, the carotenes, and xanthophylls. Recently selenium, at levels of 0.01

to 0.1 p.p.m., has been shown to prevent or cure exudative diathesis in vitamin E-deficient chicks (Scott *et al.*, 1957; Schwarz *et al.*, 1957; Patterson *et al.*, 1957). Work by Scott (1962a, 1962b) shows that vitamin E plays a multiple role in the nutrition of poultry. It is required not only for normal reproduction but also (1) as nature's most effective antioxidant for prevention of encephalomalacia, (2) in a specific role, interrelated with the action of selenium, for prevention of exudative diathesis, and (3) in another role, interrelated with both selenium and cystine, for prevention of nutritional muscular dystrophy. A review of the work on selenium is contained in the previous chapter.

Chemical nature and occurrence of vitamin E. Vitamin E is the name applied to a group of fat-soluble, unstable organic compounds known as the tocopherols. d-Alpha-tocopherol possesses the greatest degree of vitamin E activity.

The tocopherols are found in the germ oils of seeds such as wheat, corn, and soybeans, and in alfalfa meal. Commercial vitamin E supplements are produced by molecular distillation of the alpha-tocopherol from vegetable oils or by chemical synthesis of *dl*-alpha-tocopherol.

Since esterification of the vitamin improves its stability, the commercial supplements usually contain *d*-alpha-tocopheryl acetate or *dl*-alpha-tocopheryl acetate. One International Unit of vitamin E is equivalent to the activity of one mg. of *dl*-alpha-tocopheryl acetate. Because the *l*-isomer is less active, *d*-alpha-tocopheryl acetate possesses an activity of 1.36 I.U. per mg. Unesterified *dl*-alpha tocopherol has a potency of 1.1 I.U., while that of *d*-alpha-tocopherol is 1.49 I.U. per mg.

Vitamin E is very unstable. Its oxidative destruction is enhanced by minerals and by unsaturated fatty acids in the diet. When a diet contains fish liver oils in any quantity, an effective antioxidant also should be added in order to prevent the oils from undergoing oxidative rancidity.

Following the initial discovery by Sing-

sen *et al.* (1953) that the antioxidant diphenyl-*p*-phenylenediamine has a marked sparing effect upon vitamin E, a number of other antioxidants have been developed which also help preserve vitamins E, A, and xanthophylls in poultry feeds. Among these, the most widely used is 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin).

Vitamin E requirements of poultry. Although both selenium, in small amounts, and antioxidants are helpful in sparing the requirements for vitamin E for certain functions, it is still necessary to be certain that practical poultry rations contain sufficient amounts of vitamin E *per se*. The approximate requirements of chickens and turkeys are presented in Table 7.1.

Symptoms and Pathology

Mature chickens. No outward symptoms occur in mature chickens receiving very low levels of vitamin E over prolonged periods. Hatchability, however, is reduced markedly (Adamstone, 1931; Adamstone and Card, 1934). Embryos from hens fed vitamin E-low rations may die as early as the fourth day of incubation. Testicular degeneration occurs in males deprived of vitamin E over prolonged periods of time.

Encephalomalacia in chicks. Encephalomalacia is a nervous derangement characterized by ataxia, backward or downward retractions of the head, sometimes with lateral twisting, forced movements, increasing incoordination, a rapid contraction and relaxation of the legs, and finally complete prostration and death (Fig. 7.8). Even under these conditions, complete paralysis of the wings or legs is not observed. The deficiency usually manifests itself between the fifteenth and the thirtieth day of the chick's life, although it has been known to occur as early as the seventh day and as late as the fifty-sixth day.

Pappenheimer and Goettsch (1931) and Pappenheimer *et al.* (1939) reported that the cerebellum, the cerebral hemispheres, the medulla, and the midbrain are

FIG. 7.8 — Encephalomalacia.

affected most commonly in the order named. In chicks which are killed soon after the appearance of symptoms of encephalomalacia, the cerebellum is softened, swollen, and the meninges are edematous. Minute hemorrhages are often visible on the surface of the cerebellum. The convolutions are flattened. In some cases, as much as four-fifths of the cerebellum may be affected, while in others lesions may be so small that they cannot be recognized grossly. A day or two after the symptoms of encephalomalacia are first manifested, the necrotic areas present a greenish-yellow opaque appearance. Healing sometimes occurs spontaneously, in which case the affected areas are shrunken and depressed below the surface of the healthy tissue, and the color changes to brownish-yellow.

In the cerebrum, the necrotic tissue is frequently pale, swollen, and wet, and in the early stages becomes sharply delineated from the remaining normal tissue (Pappenheimer et al., 1939). Some cases are so affected that the greater portion of both hemispheres in the cerebrum are destroyed. Other cases are so mildly affected that lesions are apparent only on microscopic examination. In the cerebrum also the affected tissue is greenish-yellow, but when healing has occurred, the color changes to a rusty brown.

Medullary lesions are not so readily noted in a macroscopic examination (Pappenheimer et al., 1939). A flattening and general swelling of the ventral surface indicates the presence of internal lesions. After one is familiar with the disease, a macroscopic diagnosis can be made correctly in approximately 90 per cent of the cases.

The microscopic lesions of the cerebellum are characteristic, but are variable in extent and distribution (Wolf and Pappenheimer, 1931; Pappenheimer et al., 1939; and Scott and Stoewsand, 1961). In all cases, edema in the beginning is followed by capillary hemorrhages, thrombosis, and necrosis of neuroglial elements and ganglion cells. Edema is probably the most constant and striking feature; it results in structural changes in the convolutions and obliteration of the sulci. Edema is apparent in the Purkinje cell zone and results in a cribriform structure. The Purkinje cells, Golgi cells, and small cells of the granular layer undergo a degeneration known as ischemic necrosis (Fig. 7.9). The Purkinje cells become angular and narrow and lose their Nissl substances; their nuclei become pyknotic. Nuclei of the Bergmann cells are swollen.

One of the earliest recognizable lesions of encephalomalacia is a circulatory disturbance in the brain (Wolf and Pappenheimer, 1931; Pappenheimer et al., 1939). The pial vessels and the capillaries of the granular and molecular layers, and the cap-

FIG. 7.9 — Encephalomalacia in the chicken. (Above) normal brain shows well-defined row of Purkinje cells between granular and molecular layers of cerebellum. ×300. (Below) Brain of chicken with encephalomalacia shows pyknosis and destruction of Purkinje cells and separation of granular and molecular layers of cerebellum. ×600. (Scott and Stoewsand, 1961)
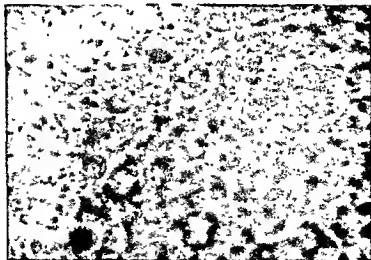
ries of the central white matter in the ·' of the cerebellum are engorged with ,throcytes. Not all vessels are distended · manner; some vessels are distended thile others are collapsed. This is particu- '; true in the white matter, but less often the granular layers, and is only occasion- lly noted in the lower portion of the mo- 'ecular layer. Large numbers of small nemorrhages are found in the pia and cere- bellar layers and are very conspicuous in the lower portion of the molecular layer. The fibers of the white matter are sepa- rated by a mild edema. Hyaline capillary thrombi appear even in the most recent and small areas of necrosis and are a very constant feature of the lesions. When the capillaries undergo repair, the endothelial cells become greatly swollen and grow very actively; they quite often sprout laterally into the necrotic tissues. The lesions in the cerebrum, midbrain, and medulla are very much like those of the cerebellum. Cere- bral lesions appear to result from vascular disturbances, but the vascular disturbances are not associated with significant alter- ations in cell plasma ratio, plasma, or blood volume (Pappenheimer and Graff, 1932; and Pappenheimer et al., 1939).

Exudative diathesis in chicks. Exudative diathesis is an edema of the subcutaneous tissues associated with abnormal perme- ability of the capillary walls (Dam and Glavind, 1939). In severe cases, the chicks stand with their legs far apart as a result of the accumulation of the fluid under the ventral skin. This greenish-blue, viscous fluid is easily seen through the skin, since it usually contains some blood components arising from slight hemorrhages which appear throughout the breast and leg mus- culature and in the intestinal walls. Dis- tension of the pericardium and sudden deaths have been noted. The onset of the condition coincides with the appearance of peroxides in the tissues.

Chicks suffering from exudative diathesis show a fairly severe microcytic anemia (Scott et al., 1955), a low ratio of albumin to globulins (Goldstein and Scott, 1956), and a low muscle creatine.

Muscular dystrophy in chickens, duck- lings, and turkeys. When vitamin E de- ficiency is accompanied by a sulfur amino acid deficiency, chicks show symptoms of muscular dystrophy, particularly of the breast muscle, at about 4 weeks of age. The condition is characterized by light colored streaks of easily distinguished affected bundles of muscle fibers in the breast (Fig. 7.10). A similar dystrophy occurs in vita- min E deficient ducks where it is general throughout all skeletal muscles of the body. Upon histological examination, and the intramuscular tissue is edematous, and the muscle fibers are seen to have undergone hyaline degeneration, leaving only masses of cell nuclei apparent in some areas. Vita- min E and selenium deficiency in chickens and, especially, in turkeys, may result in an extreme dystrophy of the gizzard muscle (Salisbury et al., 1962; Walter and Jensen 1963). According to Walter and Jensen (1963) this gizzard dystrophy in turkeys is prevented by supplementing deficient diets with either vitamin E or selenium. It is not affected by the dietary level of sulfur amino acids.

Enlarged hock disorder in turkeys. Turkeys receiving low vitamin E diets, con- taining 2 per cent level of cod liver oil, develop characteristic hock en- largements and bowed legs at approxi- mately 2 to 3 weeks of age (Scott, 1951). As the poults are allowed to continue to grow on these diets, the hock enlargements usually disappear by the time the poults are 6 weeks of age, only to reappear in more severe form when the turkeys reach 14 to 16 weeks of age, especially in toms raised on wire or slat floors. Creatine ex- cretion is increased and muscle creatine levels are reduced.

The development of enlarged hocks ap- pears to be a weakness in turkeys which may result from a number of deficiencies and stresses. In addition to the need for adequate vitamin E, it appears that phos- phorus, choline, glycine, niacin, zinc, and unknown factors are needed in adequate amounts for the prevention of the enlarged

FIG. 7.10 — Nutritional muscular dystrophy in the chicken. White striations are degenerated muscle fibers clearly visible in the muscles of the breast and leg.

hock disorder (Slinger *et al.*, 1954; Scott, 1950; Scott, 1953). Two infectious diseases, synovitis and staphylococcic arthritis, may show somewhat similar symptoms.

Treatment of vitamin E deficiency. Symptoms of exudative diathesis and muscular dystrophy in chicks are readily reversed, if not too far advanced, by administration of vitamin E, either by oral dosing or in the feeds. Oral administration of a single dose of 300 I.U. of vitamin E per chick caused remission of exudative diathesis and maintained the chicks in normal condition for about one week after dosing (Goldstein and Scott, 1956). Encephalomalacia may or may not respond to treatment with vitamin E, depending upon the extent of the damage to the cerebellum.

VITAMIN K AND VITAMIN K DEFICIENCY

Vitamin K is required for the synthesis of prothrombin within the body. Since prothrombin is an important part of the blood-clotting mechanism, a deficiency of vitamin K results in a markedly prolonged blood-clotting time, such that an affected chick or poult may bleed to death from a slight bruise or other injury.

Chemical nature and occurrence. Two forms of vitamin K, vitamin K_1 and vitamin K_2, exist in nature. Each has a 2-methyl-1,4-naphthoquinone nucleus with a side chain. Vitamin K_1 has a phytyl side chain made up of four isoprene units with the empirical formula of $C_{20}H_{31}OH$; vitamin K_2 has a difarnesyl side chain of six isoprene units, with the empirical formula of $C_{30}H_{49}OH$. Many synthetic 2-methyl-1,4-naphthoquinones have variable amounts of vitamin K activity, depending upon the makeup of the side chain. Synthetic vitamin K (K_3 or menadione), however, has a single hydrogen atom in place of the side chain.

The richest sources of vitamin K (K_1) are the green leaves of plants such as

a and green grasses. However, soybean oil also contains this vitamin. Vitamin K_2 is synthesized by a number of bacteria, and, therefore, is present in materials which have promoted the growth of these bacteria. This form of the vitamin has been isolated from fish which has been subjected to bacterial action. However, because of improved processing, present-day fish meal may not represent a dependable source of the vitamin. Although vitamin K_2 is synthesized in the intestinal tracts of chickens and turkeys, it appears too far down the tract to furnish these species with all of their vitamin K needs.

Requirements. The vitamin K_1 requirement of young chicks is presented in Table 7.1. This requirement is based upon practical diets in the absence of stress agents, such as sulfaquinoxaline, which may increase the requirement. When sulfaquinoxaline or other drugs are present in the feed or in the drinking water, supplementary menadione sodium bisulfite is usually added at levels of 2 to 3 gm. per ton of feed.

Symptoms and Pathology

Symptoms of vitamin K deficiency occur most frequently about 2 to 3 weeks after chicks are placed on a vitamin K-deficient diet. The presence of sulfaquinoxaline in the feed or in the drinking water may increase the incidence and severity of the symptoms. Large hemorrhages appear on the breast, legs, wings, and/or in the abdominal cavity. The chicks show an anemia which may be in part due to the loss of blood but also due to the development of a hypoplastic bone marrow. Although blood-clotting time is a fairly good measure of vitamin K deficiency, a more accurate measure is obtained by determining the "prothrombin-time."

Treatment. Within 4 to 6 hours after vitamin K is administered to deficient chicks, the blood clots normally, but recovery from the anemia or disappearance of the hemorrhages cannot be expected to take place promptly. Failure to realize this may account for the apparent failure of recovery from some cases of "field hemorrhagic syndrome" upon treatment with menadione sodium bisulfite.

THIAMINE (VITAMIN B_1) AND THIAMINE DEFICIENCY

Thiamine is required by poultry for the metabolism of carbohydrates. In the body, it becomes an important part of the enzyme, carboxylase, which is concerned in a number of reactions involving pyruvate, one of the end products of carbohydrate catabolism. Deficiency of thiamine in poultry leads to extreme loss of appetite, polyneuritis, and death.

Chemical nature and occurrence. Thiamine is a water-soluble, heat-unstable compound consisting of a thiazole ring attached to a pyrimidine nucleus by a methylene linkage. The thiazole moiety contains a two carbon side chain with a terminal primary alcohol grouping, which is the site of attachment of phosphoric acid in the formation of cocarboxylase.

The cereal grains and their by-products, soybean meal, cottonseed meal, peanut meal, and alfalfa meal are all relatively rich sources of thiamine. Thus, under normal circumstances, all practical poultry rations contain adequate thiamine without the addition of special feed supplements high in thiamine.

Under certain conditions, however, a thiamine deficiency in a poultry feed may be created. Thiamine is very unstable to be created. Thiamine is very unstable to heat under neutral and alkaline conditions. Tests show that while no destruction occurs in 1 per cent HCl during 7 hours at 100° C., 96.4 per cent destruction occurs under the same conditions at pH 7, and 100 per cent destruction occurs in 15 minutes at pH 9 at 100° C. Poultry rations, therefore, especially pelleted rations, should not contain alkaline salts in sufficient quantities to produce an alkaline reaction in the feed. Bisulfite ions are also very destructive of thiamine, cleaving the molecule into the pyrimidine and thiazole

parts. An enzyme called thiaminase, capable of destroying thiamine, exists in fresh fish (Green et al., 1912) and in other materials, including the heart and spleen of warm-blooded animals (Somogyi, 1952). Oxythiamine is a potent antagonist of thiamine (Daniel and Norris, 1949). When this compound is present in the diet, much more thiamine is required to prevent a deficiency. According to Bhagvat and Devi (1944), many natural feedstuffs, such as beans and mustard seed, contain antithiamine-active compounds.

Requirements. The thiamine requirement of young chicks is shown in Table 7.1. This requirement is easily met by most practical feeds.

The thiamine requirements of breeding hens and of starting and breeding turkeys also are easily met by practical feedstuffs without special supplementation.

Symptoms and Pathology

Thiamine deficiency or polyneuritis, as it is usually called, is observed in mature chickens approximately 3 weeks after they are placed on a thiamine-deficient diet. In young chicks, it may appear before 2 weeks of age. The onset of symptoms is sudden in young chicks and more gradual in mature birds. Anorexia is the first symptom, followed by loss in weight, ruffled feathers, leg weakness, and an unsteady gait. Adult chickens often show a blue comb. As the deficiency progresses, apparent paralysis of the muscles occurs, beginning with the flexors of the toes and progressing upward, affecting the extensor muscles of the legs, wings, and neck. The chicken, characteristically, sits on its flexed legs and draws the head back in a "star-gazing" position (Fig. 7.11). Retraction of the head is due to paralysis of the anterior muscles of the neck. Soon after this stage, the chicken loses the ability to stand or sit upright and topples to the floor, where it lies with the head still retracted in many instances.

The body temperature drops to as low as 96° F. (Vedder and Clark, 1912). A progressive decrease in the respiratory rate

FIG. 7.11 — Typical "star-gazing" pose displayed by chick suffering from thiamine deficiency.

occurs. The adrenal glands hypertrophy more markedly in females than males. The cortex is apparently affected to a greater extent than the medulla. Apparently the degree of hypertrophy in the adrenals determines the degree of edema of the tissues (McCarrison, 1918). In the chicken, edema occurs largely in the skin (Krause, 1922). It has been observed that the epinephrine content of the adrenals increases as this organ hypertrophies. Atrophy of the genital organs also occurs in chickens affected with thiamine deficiency. This is more pronounced in the testes than in the ovaries. The heart shows a slight degree of atrophy. The right side of the heart is frequently dilated, the auricle being more frequently affected than the ventricle. Atrophy in the stomach and intestinal walls may be sufficiently severe to be noted without the aid of a microscope.

Treatment. Chickens suffering from thiamine deficiency respond, in a matter of a few hours, to oral administration of the vitamin. Since thiamine deficiency causes extreme anorexia, supplementing the feed with the vitamin is not a reliable treatment until after the chickens have recovered from the acute deficiency via oral administration of thiamine.

RIBOFLAVIN (VITAMIN B₂) AND RIBOFLAVIN DEFICIENCY

Riboflavin forms the active part of over a dozen enzyme systems in the body. The

noted of these is the "yellow oxi-
dase enzyme." Other important ribo-
flavin containing enzymes are: cytochrome
reductase, diaphorase, xanthine oxidase,
L- and D-amino acid oxidases, and hista-
minase, all of which are vitally associated
with the oxidation-reduction reactions in-
volved in cell respiration.

Chemical nature and occurrence. Ribo-
flavin is a heat-stable, water-soluble com-
pound, containing an isoalloxazine nucleus
and a ribose side chain. It is subject to
destruction by light, especially in alkaline
solution. It is normally a yellow substance,
solutions of which give off a greenish
fluorescence when exposed to blue or ultra-
violet light. Treatment of solutions of
riboflavin with reducing agents converts it
to the colorless form (leuco-form), which
shows no fluorescence. The greenish color
of egg albumen is due to the riboflavin
present in it. Riboflavin is present in milk
by-products, alfalfa, grass meals, and liver.
It is synthesized by yeasts and bacteria.
Riboflavin for commercial feed supple-
ments is produced by chemical synthesis
and by fermentation of waste materials
with various bacteria, especially *Clostrid-
ium acetobutylicum.*

Requirements. The riboflavin require-
ments of chickens and turkeys are pre-
sented in Table 7.1. The requirements of
chickens were confirmed by Hill et al.
(1954).

Symptoms and Pathology

Symptoms of riboflavin deficiency in the
chick were first reported by Norris et al.
(1930), Bethke et al. (1931), and Lepkov-
sky and Jukes (1936). When chicks are
fed a diet deficient in riboflavin, they grow
very slowly, become weak and emaciated;

their appetite is fairly good, and diarrhea
develops between the first and second
week. The chicks do not walk except when
forced to do so, and then frequently walk
upon their hocks with the aid of their
wings. The toes are curled inward (Fig.
7.12), both when walking and when rest-
ing on their hocks. The chicks are usually
found in a resting position. The wings
often droop as though it were impossible
for the chicks to hold them in the normal
position. The leg muscles are atrophied
and flabby, and the skin is dry and harsh.
Young chicks in advanced stages of defi-
ciency do not move around but lie with
their legs sprawled out.

Postmortem examination does not show
any marked abnormalities of the internal
organs, nor does bacteriological exami-
nation reveal any specific infection of the
blood or other internal organs. In some
cases, the thymus shows congestion and pre-
mature atrophy.

According to Davis et al. (1938a,
1938b) and Lepkovsky et al. (1938), the
only symptoms noted in a deficiency of
riboflavin in the diet of hens are decreased
egg production, increased embryonic mor-
tality, and an increase in the size and the
fat content of the liver. The hatchability
of eggs becomes poor within 2 weeks after
hens are fed a riboflavin-deficient diet,
but the hatchability improves to nearly
normal within 7 days after adequate
amounts of riboflavin are added to the
diet. The embryos which fail to hatch
from the eggs of hens on diets low in this
vitamin are dwarfed, and show a high in-
cidence of edema, degeneration of the
Wolffian bodies, and a characteristically
defective down. This type of down is re-
ferred to as "clubbed," and results from a

FIG. 7.12 — Riboflavin deficiency
(curly toe).

failure of the down feathers to rupture the sheaths; this causes the feathers to coil and take the shape of a French knot.

Riboflavin deficiency in the young turkey is characterized by poor growth and incrustations in the corners of the mouth and on the eyelids. Severe dermatitis of the feet and shanks, marked by edematous swelling, desquamation, and deep fissures, appears in some of the deficient poults (Heuser, 1935; Bethke and Record, 1942; McGinnis and Carver, 1947). It is noted that these symptoms of riboflavin deficiency in the turkey are similar to those of pantothenic acid deficiency in the chicken.

In severe cases of riboflavin deficiency, chicks show a very marked swelling and softening of the sciatic and brachial nerves. The sciatic nerves usually show the most pronounced effects. They may reach a diameter 4 to 5 times the normal size.

Phillips and Engel (1938) reported that histologic examinations of the affected nerves show definite degenerative changes in the myelin sheaths of the main peripheral nerve trunks. This may be ac-

companied by axis cylinder swelling and fragmentation, Schwann cell proliferation, myelin changes, gliosis, and chromatolysis in the spinal cord. In cases of curled-toe paralysis, degeneration of the neuro-muscular end plate and muscle tissues is often found. This indicates that riboflavin is necessary for the normal functioning of the nervous system of the growing chick. Riboflavin is probably also essential for myelin metabolism of the main peripheral nerve trunks. No gross dystrophy develops, although muscle fibers are, in some cases, completely degenerated. The sciatic nerve exhibits myelin degeneration in one or more of its branches. Similar changes are apparent in the brachial nerve trunks.

In the case of embryos which fail to hatch from eggs laid by hens fed riboflavin-deficient diets, the nervous system shows degenerative changes very much like those described in riboflavin-deficient chicks (Engel et al., 1940).

Treatment of deficiency. Chicks receiving rations only partially deficient in riboflavin may recover spontaneously, indi-
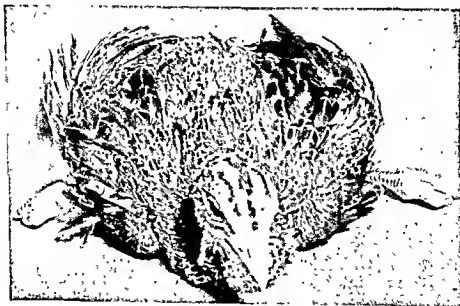
FIG. 7.13 — A 35-day-old poult showing riboflavin deficiency with curled-toe paralysis. (Richardson, Tex. Agr. Exper. Sta.)
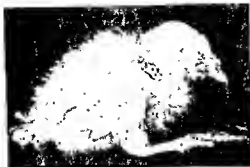
FIG. 7.15 — Dermatosis of pantothenic acid deficiency in the chick.

and severe edema are symptoms of pantothenic acid deficiency in the developing chick embryo (Beer et al., 1963).

The symptoms of pantothenic acid deficiency in chicks are difficult to differentiate from those of biotin deficiency. Robblee and Clandinin (1950) reported that deficiencies of pantothenic acid and biotin result in dermatitis, broken feathers, perosis, poor growth, and mortality. Pantothenic acid deficient chicks are characterized by retarded and rough feather growth (Norris and Ringrose, 1930). The chicks are emaciated, and definite crusty scablike lesions appear in the corners of the mouth. The margins of the eyelids are granular, and small scabs develop on them. The eyelids are frequently stuck together by a viscous exudate; they are contracted, and vision is restricted (Fig. 7.15). In some cases the feathers are lost from the head. There is a slow sloughing of the keratinizing epithelium of the skin. The outer layers of skin between the toes and on the bottoms of the feet sometimes peel off, and small cracks and fissures appear at these points. These cracks and fissures enlarge and deepen, and the chicks move about very little. In some cases the skin layers of the feet of deficient chicks thicken and cornify, and wartlike protuberances develop on the balls of the feet.

Postmortem examination shows the presence of a puslike substance in the mouth and an opaque, grayish-white exudate in the proventriculus (Ringrose et al., 1931). The liver is hypertrophied and may vary in color from a faint yellow to a dirty yellow. The spleen is slightly atrophied. The kidneys are somewhat enlarged. The nerves and myelinated fibers of the spinal cord show myelin degeneration (Phillips and Engel, 1939). These degenerating fibers occur in all segments of the cord down to the lumbar region.

Gillis et al. (1948) showed that the amount of pantothenic acid in the feed for a hen has a definite effect on the hatchability of the eggs produced. Embryonic mortality was high when the hens were fed a diet low in pantothenic acid. Most of the mortality occurred during the last 2 or 3 days of the incubation period.

Treatment of deficiency. The symptoms of pantothenic acid deficiency appear to be completely reversible, if not too far advanced, by oral treatment or injection with the vitamin, followed by restoration of an adequate level (Table 7.1) of pantothenic acid in the diet.

Pantothenic acid deficiency, in sufficient severity to cause characteristic symptoms of the deficiency, has not been demonstrated to occur under field conditions. Robertson et al. (1949) noted that the disease known as "stunted chick disease" exhibits many of the symptoms characteristic of pantothenic acid deficiency. However, substitution of 5 per cent liver meal for 5 per cent meat scrap in the ration or injection of the chicks with 100 μg. of pantothenic acid failed to provide any protection in the course of the disease.

On the other hand, another field disease has been observed which may indicate a deficiency of pantothenic acid in chicken breeder rations under certain conditions. In this disease, first noted by Hill (1954), egg production and hatchability of fertile eggs are normal, but the chicks are underweight, weak, and may suffer up to 50 per cent mortality during the first 24 hours after hatching. Hill (1954) and Fisher and Hudson (1956) found that injection of the chicks with a mixture of B vitamins, or with pantothenic acid alone,

mature poultry with niacin, and no deficiency symptoms have been described.

Treatment. Supplementation of a deficient ration with the required amounts of niacin, as shown in Table 7.1, usually brings about rapid recovery from all symptoms of deficiency, including the hock enlargements and bowed legs, in young chicks, ducklings, or poults. Niacin supplementation usually has little or no effect upon cases which have progressed to the extent that the tendon has slipped from its condyles (perosis), or upon advanced cases of "enlarged hock disorder" in adult tom turkeys.

PYRIDOXINE (VITAMIN B₆) AND PYRIDOXINE DEFICIENCY

Pyridoxine is required in several enzymes, particularly those concerned with deamination, transamination, and decarboxylation of amino acids. The coenzymes for these reactions are known as pyridoxal phosphate and pyridoxamine phosphate.

Chemical nature and occurrence. Pyridoxine, like niacin, is a stable member of the vitamin B complex containing a pyridine nucleus and a primary alcohol grouping. Pyridoxal and pyridoxamine are derivatives of pyridoxine containing an aldehyde and amine group, respectively, instead of the alcohol group. Most feedstuffs are fairly good sources of pyridoxine (Schneider et al., 1939), thereby making it unnecessary to supplement practical poultry rations with this vitamin.

Requirements. Relatively little research work has been done on the pyridoxine requirements of poultry. The requirements based upon published work are shown in Table 7.1. A number of studies of pyridoxine requirements have been conducted by Fuller and Kifer, 1959; Fuller and Dunahoo, 1959; and Fuller et al., 1961. Lucas et al. (1946) found that crossbred chicks (Rhode Island Red × Barred Plymouth Rock) showed a considerably higher requirement for pyridoxine than had previously been found by Hogan et al.

(1941) and by Briggs et al. (1942b) for White Leghorn chicks. This suggests that the pyridoxine requirement may vary with different breeds and families or, as indicated under the discussion of the niacin-tryptophan-pyridoxine interrelationship, may depend upon the levels of certain other nutrients in the diet.

Symptoms

Pyridoxine deficient chicks show depressed appetite, poor growth, and characteristic nervous symptoms. The chicks show jerky, nervous movements of the legs when walking and often undergo extreme, spasmodic convulsions which usually terminate in death. During these convulsions, chicks may run aimlessly about, flapping their wings and falling to their sides or rolling completely over on their backs, where they perform rapid jerking motions with their feet and heads. These symptoms may be distinguished from those of encephalomalacia by the relatively greater intensity of activity of the chicks during a seizure resulting from pyridoxine deficiency, which results in complete exhaustion and often in death.

In adult birds, pyridoxine deficiency causes marked reduction of egg production and hatchability as well as decreased feed consumption, loss of weight, and death (Cravens et al., 1943, 1946).

BIOTIN AND BIOTIN DEFICIENCY

Although biotin is a dietary requirement and a deficiency of this vitamin produces very severe lesions, no definite metabolic role has been established for biotin in poultry.

Chemical nature and occurrence. Biotin is a sulfur-containing, complex member of the vitamin B complex. It is present in common feedstuffs in sufficient amounts to meet the biotin requirements of poultry without the use of special biotin-rich supplements. Liver meal, yeast, milk byproducts, and molasses are rich sources.

Unheated egg white contains a protein, avidin, which is capable of reacting with

FIG. 7.16 — A biotin-deficient chick.

biotin in the intestinal tract of chicks or other animals, rendering the dietary biotin unavailable to the animal and thereby creating a biotin deficiency. Thus, only under the unusual conditions in which raw egg white is used in a poultry ration, is a deficiency of biotin likely to be encountered.

Requirements. Only the requirement for starting chicks has been studied. This is given in Table 7.1.

Symptoms and Pathology

In biotin deficiency, the dermatitis of the feet and the skin around the beak and eyes (Fig. 7.16) is similar to that described under the section on pantothenic acid. Thus, in making a differential diagnosis between biotin and pantothenic acid deficiency, it is usually necessary to examine the composition of the ration fed and decide which vitamin is more likely to be deficient in the ration. This can be checked by feeding the ration to two groups of chicks, supplementing the feed for one group with biotin, the other with pantothenic acid.

Couch et al. (1948) reported that congenital perosis, ataxia, and characteristic skeletal deformities developed when the hens were fed a low-biotin diet which did not favor intestinal synthesis of the vitamin. The deformities were prevented by adding biotin to the diet. These embryonic deformities consisted of a shortened tibiotarsus which was bent posteriorly, a much shortened tarsometatarsus, shortening of the bones of the wing and of the skull, and shortening and bending of the anterior end of the scapula.

According to Cravens et al. (1942, 1944), embryos from hens fed biotin-deficient diets developed syndactylia, an extensive webbing between the third and fourth toes. These workers also observed that a large number of the embryos which failed to hatch were chondrodystrophic, and were characterized by a reduced size, a parrot beak, severely crooked tibia, and/or a much shortened or twisted tarsometatarsus. One peak of embryonic mortality occurred during the first week of incubation, a second during the last 3 days.

Perosis is also a characteristic deficiency symptom of biotin avitaminosis. This has been demonstrated by a number of workers including McElroy and Jukes (1940), Jukes and Bird (1942), and Richardson et al. (1942). Symptoms of perosis are described in Chapter 6 under Manganese.

Treatment. If biotin deficiency is observed in a poultry flock, one should first investigate the possibility of the presence of uncooked egg white in the diet, since biotin deficiency is very difficult to produce, even with highly purified feed ingredients, unless raw egg white (avidin) is added to the diet. Biotin supplementation of a diet containing raw egg white might not correct the deficiency unless the level of biotin used is in excess of the biotin-combining capacity of the egg white. Under these conditions, injection of a few micrograms of biotin should produce recovery, which should be maintained by exclusion of egg white from the diet.

FOLIC ACID (FOLACIN) AND FOLIC ACID DEFICIENCY

Folic acid is a part of the enzyme systems concerned in "single carbon" metabo-

lism. In this manner, it is involved in the synthesis of purines and of the methyl groups of such important metabolites as choline, methionine, and thymine. Because of this, folic acid is required for normal nucleic acid metabolism and for the formation of the nucleo-proteins required for cell multiplication.

Chemical nature and occurrence. Folic acid is a fairly stable member of the vitamin B complex. Its chemical structure contains three distinct parts: (1) a pterin (related to xanthopterin, the pigment in yellow butterfly wings), (2) *p*-amino benzoic acid (PABA), and (3) glutamic acid. The chemical name for folic acid is pteroylglutamic acid. An important derivative of folic acid is the citrovorum factor (leucovorin or folinic acid), which resembles folic acid except that it contains four additional hydrogen atoms and a formyl group. The research evidence indicates that the citrovorum factor is the metabolically active form of folic acid. Much of the folic acid in natural feedstuffs is conjugated with varying numbers of extra glutamic acid molecules. In these bound forms, it is inactive for the assay microorganisms and as a metabolite in the animal body. However, chicken and turkey pancreas, liver, and kidney contain enzymes capable of releasing free folic acid and free citrovorum factor from the respective conjugates. The activities of these "conjugases" are influenced by a number of other factors (Hill and Scott, 1951, 1952a, 1952b).

Yeast, liver, alfalfa meal, and soybean meal are rich sources of folic acid, while corn is a relatively poor source. The high level of soybean meal in practical poultry rations is largely responsible for the fact that these rations need not be supplemented with special sources of this vitamin. Bearse *et al.* (1955b) found that with corn as the cereal portion of a chick starter ration, chicks developed symptoms of folic acid deficiency when soybean meal was replaced with herring meal as the protein supplement. The deficiency was prevented

either by substitution of soybean meal for herring meal, by substitution of milo for corn, or by supplementing the herring meal-corn ration with folic acid.

Requirement. The results of studies on the folic acid requirements of chickens and poults are summarized in Table 7.1.

Symptoms and Pathology

Folic acid deficiency in chicks is characterized, grossly, by poor growth, very poor feathering, an anemic appearance (Robertson *et al.*, 1946), and perosis (Daniel *et al.*, 1946). McGinnis *et al.* (1942) discovered that a factor, later shown by Frost *et al.* (1946) to be the *L. casei* factor (folic acid), is required for pigmentation in the feathers of Rhode Island Red and Black Leghorn chicks. Thus, folic acid, lysine, and iron (see Chapter 6) appear to be required for prevention of white feathering in colored poultry. A deficiency in the breeding ration causes a marked increase in embryonic mortality. The embryos usually die soon after pipping the air cell. According to Sunde *et al.* (1950a, 1950b), a deformed beak and bending of the tibiotarsus are symptoms of the embryonic deficiency. Poults show a characteristic "cervical paralysis" (Fig. 7.17), and die within 2 days after the onset of these symptoms unless folic acid is administered immediately. Poults show only a slight anemia.

Folic acid deficiency in chicks results in megaloblastic arrest of erythrocyte formation in the bone marrow, which results in a severe macrocytic anemia as one of the first symptoms in chicks. White cell formation is also reduced, which causes a marked agranulocytosis. Further evidence that folic acid is necessary for cell mitosis is supplied by these discoveries: (1) that oviduct growth is not increased in estrogen-treated chicks unless the diet is supplemented with folic acid (Hertz, 1945; Haque *et al.*, 1949) or, preferably, with the citrovorum factor (Kline and Dorfman, 1951); (2) that the growth of the chicken embryo is inhibited by very

FIG. 7.17 — Folic acid deficiency, showing "straight neck" paralysis. (Richardson and Hogan, Ma. Agr. Exper. Sta.)

small amounts of the folic acid antago-nist, 4-aminofolic acid (Karnofsky *et al.*, 1949), the inhibition being reversed by adding citrovorum factor (Cravens and Snell, 1950); and (3) that Rous chicken sarcoma is inhibited completely either by folic acid deficiency or by feeding 4-amino-folic acid (Little *et al.*, 1948).

Folic acid-choline interrelationship. In the presence of adequate folic acid in the diet, only approximately 260 mg. of cho-line are required to prevent perosis in chicks. But when the diet is deficient in folic acid, an increase in the level of choline causes some improvement in the incidence and severity of perosis, whereas levels up to 900 mg. of choline per pound of diet fail to prevent this disorder com-pletely (Young *et al.*, 1955).

Treatment. A single intramuscular in-jection of 50 or 100 µg. of pure pteroyl-glutamic (folic) acid causes a peak reticu-locyte response (70 per cent) within 4 days in severely anemic, folic acid-deficient chicks (Robertson *et al.*, 1947). The hemo-globin values and growth rates return to normal within one week. Oral administra-tion of 50 µg. of folic acid was much less effective, whereas the addition of 500 µg. of folic acid per 100 gm. of feed caused re-

covery comparable to that obtained with injection of the vitamin.

VITAMIN B$_{12}$ (COBALAMIN) AND VITAMIN B$_{12}$ DEFICIENCY

Vitamin B$_{12}$ is concerned in nucleic acid synthesis, methyl synthesis, carbohydrate metabolism, fat metabolism, and mainte-nance of glutathione blood levels. Al-though evidence exists that vitamin B$_{12}$ is concerned in protein metabolism, Henry and Kon (1956) suggest that this is not due to an effect upon protein utilization but to the effect of this vitamin in aiding the synthesis of the methyl group needed for the formation of methionine.

Chemical nature and occurrence. Vita-min B$_{12}$ is the most recently identified member of the water-soluble, B-complex group of vitamins. It has a complicated structure of organic moieties surrounding a central cobalt atom. Vitamin B$_{12}$ con-tains a cyano group and is known chemi-cally as cyanocobalamin. Other active cobalamins contain such anions as hy-droxy, chloro, bromo, sulfato, nitro, or nitrito groups in place of the cyano group. Thus, vitamin B$_{12a}$ and vitamin B$_{12b}$ have been shown to be hydroxycobalamin, and vitamin B$_{12c}$ is nitritocobalamin.

The primary occurrence of vitamin B_{12} in nature appears to be solely the result of microbial synthesis. Little or no evidence has been obtained to show that this vitamin can be produced by higher plants, and there is no evidence of its synthesis in animal tissues. The vitamin is found in all foods of animal origin. Early recognition of this accounts for its being referred to as the "animal protein factor" (APF). The best poultry feed sources are fish solubles, fish meal, liver meal, meat scraps, and the commercial vitamin B_{12} feed supplements which are produced by special fermentations using vitamin B_{12}-synthesizing microorganisms.

Requirements. The vitamin B_{12} requirements of poultry depend upon the levels of several other nutrients in the diet. The relationship between vitamin B_{12} and pantothenic acid has been discussed earlier. Excess protein in the diet increases the need for vitamin B_{12}. The vitamin B_{12} requirement also appears to depend upon the levels of choline, methionine, and folic acid in the diet and is interrelated with ascorbic acid metabolism in the body. The vitamin B_{12} requirements of chicks and hens, under normal conditions, are shown in Table 7.1.

Symptoms and Pathology

Symptoms of vitamin B_{12} deficiency are slow growth, decreased efficiency of feed utilization, mortality, and reduced hatchability. No characteristic symptoms specific for vitamin B_{12} deficiency have been demonstrated in growing or mature poultry. Perosis may occur in vitamin B_{12}-deficient chicks or poults when the diet lacks choline, methionine, or betaine as sources of methyl groups. Addition of vitamin B_{12} may prevent perosis under these conditions, because of its effect upon the synthesis of methyl groups. Kline (1955) demonstrated that in vitamin B_{12}-deficient pullets maintained on a diet low in choline and methionine, oviduct response to treatment with diethylstilbestrol was significantly lower than in pullets receiving vitamin B_{12}. McGinnis et al. (1948) reported that a deficiency of vitamin B_{12} caused an increased nonprotein nitrogen level in the blood of chicks, which was reduced to normal by feeding a vitamin B_{12}-rich liver concentrate. Olcese et al. (1950) reported a peak in embryonic mortality at the seventeenth day of incubation of eggs from vitamin B_{12}-deficient hens. They reported a myoatrophy of the legs in the vitamin B_{12}-deficient embryos (Fig. 7.18). Other anomalies associated with vitamin B_{12} deficiency in the embryos were hemorrhages and perosis.

Treatment. Peeler et al. (1951) showed that the intramuscular injection of 2 μg. of vitamin B_{12} per hen caused the hatchability of eggs from the vitamin B_{12}-deficient hens to increase from approximately 15 per cent to 80 per cent within one week. Chin et al. (1958) found that the addition of 4 mg. of vitamin B_{12} per ton of a breeding ration was sufficient to maintain maximum hatchability and for the production of chicks having sufficient stores of vitamin B_{12} to prevent any deficiency of this vitamin during the first few weeks of life. Similar injections of young chicks, followed by a similar supplementation of the chick ration, also will correct a vitamin B_{12} deficiency in chicks. Lillie et al. (1949) showed that when eggs laid by hens deficient in vitamin B_{12} were injected with the crystalline vitamin, hatchability and subsequent growth of the chicks were improved.

CHOLINE AND CHOLINE DEFICIENCY

Choline is present in acetylcholine, in the body phospholipids, and acts as a methyl source in the synthesis within the body of methyl-containing compounds such as methionine, creatine, and N-methylnicotinamide. Acetylcholine is the chemical substance produced at the termination of the parasympathetic nerves upon stimulation and is responsible for their effects, such as the vagus inhibition of the heart. Some sympathetic nerves also are cholinergic. Choline, per se, does not act as a methyl donor, but first must be oxidized to the compound, betaine, which can

FIG. 7.18 — Embryos with myoatrophy, caused by deficiency of vitamin B₁₂ (left and right), and normal embryo (center). (Olcese, Texas A. and M. College System.)

then donate one of its three methyl groups to a methyl acceptor such as homocysteine or glycocyamine, for the formation of methionine or creatine, respectively.

Chemical nature and occurrence. Choline is N-trimethyl ethanolamine. It can be synthesized in the body of the chick by the further methylation of N-monomethyl ethanolamine. The methyl groups may arise from methionine, betaine, or may be synthesized de novo through a mechanism involving vitamin B₁₂.

Poultry feedstuffs rich in choline are liver and glandular meal, fish meal and fish solubles, yeast, distillers solubles, and soybean meal.

Requirement. The choline requirements, under normal conditions, are presented in Table 7.1.

Symptoms and Pathology

In addition to poor growth, the outstanding symptom of choline deficiency in chicks and poults is perosis. Perosis is first characterized by pinpoint hemorrhages and a slight puffiness about the hock joint. This is followed by an apparent flattening of the tibiometatarsal joint which is caused by a rotation of the metatarsus (Milne,

1936). The metatarsus continues to twist and may become bent or bowed so that it is out of alignment with the tibia. When this condition exists, the leg cannot adequately support the weight of the bird. The articular cartilage is displaced, and the tendon of Achilles slips from its condyles.

According to Abbott and DeMasters (1940), when laying pullets are fed diets deficient in choline, increased mortality, increased abortion of egg yolks from the ovaries, and an increase in the percentage of fatty acids in the liver result. The percentage of fatty acids in the livers of choline-deficient chickens is much higher in females than in males. However, other evidence shows that choline deficiency is rare in adult chickens and turkeys fed practical rations.

Treatment. If a diagnosis of choline deficiency is noted in chicks or poults before the severe symptoms of perosis have developed, the deficiency can be cured by supplementing the ration with sufficient choline to meet the requirements indicated in Table 7.1. Once the tendon has slipped in chicks or poults suffering from choline deficiency, the damage is irreparable.

VITAMIN C, INOSITOL, p-AMINOBENZOIC ACID AND LIPOIC ACID

Vitamin C (ascorbic acid) is synthesized in adequate amounts by all species of poultry (Carrick and Hauge, 1925; Hart et al., 1925b).

Inositol has been known for years to be an essential nutrient for certain yeasts and other microorganisms. Evidence from a number of studies indicates that inositol is not required in the diet of poultry.

p-Aminobenzoic acid is a part of the folic acid molecule. Thus, the presence of this compound in the diet aids the in-

testinal microorganisms in their synthesis of folic acid, which could benefit the chicken or turkey if the diet happened to be deficient in folic acid.

Lipoic acid (also referred to as protogen or thioctic acid) is a newly discovered metabolite concerned in oxidative decarboxylation in carbohydrate (pyruvate) metabolism. Lipoic acid is apparently synthesized in adequate amounts by the chick. Stokstad et al. (1953), Supplee et al. (1956), Morrison and Norris (1956), and others have shown that the addition of lipoic acid to purified diets for chicks fails to cause any improvements in growth.

REFERENCES

Abbott, O. D., and DeMasters, C. U.: 1940. Choline in the diet of chickens. Jour. Nutr. 19.47.

Ackert, J. E., McIlvaine, M. F., and Crawford, N. Z.: 1931. Resistance of chickens to parasitism affected by vitamin A. Am. Jour. Hyg. 13.320.

Adamstone, F. B.: 1931. The effects of vitamin E deficiency on the development of the chick. Jour. Morph. and Physiol. 52:47.

———: 1947. Histologic comparison of the brains of vitamin A-deficient and vitamin E-deficient chicks. Arch. Path. 43.301.

———, and Card, L. E.: 1934. The effects of vitamin E deficiency on the testis of the male fowl (Gallus domesticus). Jour. Morph. 56:339.

Armininton, M., Heal, H. M., and Sullivan, T. W.: 1964. The young turkey's requirement for pyridoxine and thiamine. Poultry Sci. 43:1301.

Baird, F. D., Ringrose, A. T., and MacMillan, N. J.: 1939. The stability of vitamins A and D in mixed feed ingredients. Poultry Sci. 18.35.

Beach, J. R.: 1924. Studies on nutritional disease of poultry caused by vitamin A deficiency. Calif. Agr. Exper. Sta. Bul. 378.

Bearse, G. E., McClary, C. F., and Saxena, H. C.: 1953a. Blood spot incidence and the vitamin A level of the diet. Poultry Sci. 32 888. (Abst.)

———, Saxena, H. C., McClary, C. F., Blaylock, L. G., and Berg, L. R.: 1953b. Deficiency of folic acid in rations containing natural feedstuffs. Poultry Sci. 32 889. (Abst.)

Beer, A. E., Scott, M. L., and Nesheim, M. C.: 1963. The effect of a deficiency of pantothenic acid on the breeding performance of White Leghorn chickens. Brit. Poultry Sci. 4.243.

Belin, S. A., Hartley, D. C., Cramer, J. W., Phillips, V. J., and Steenbock, H.: 1954. The effect of vitamin D on urinary citrate in relation to calcium, phosphorus and urinary pH. Arch. Biochem. and Biophys. 50:18.

Bessey, O. A., and Wolbach, S. B.: 1939. Vitamin A physiology and pathology. The Vitamins, Am. Med. Assn., Chicago, Ill., p. 27

Bethke, R. M., and Record, P. R.: 1942. The relation of riboflavin to growth and curled-toe paralysis in chicks. Poultry Sci. 21:147.

———, Record, P. R., and Kennard, D. C.: 1931. A type of nutritional leg paralysis affecting chicks. Poultry Sci. 10.355.

———, Record, P. R., Kick, C. H., and Kennard, D. C.: 1936. Effect of different sources of vitamin D on the laying bird. I. Egg production, hatchability and tissue composition. Poultry Sci. 15.326.

Bhagvat, K., and Devi, P.: 1944. Inactivation of thiamine by certain foodstuffs and oil seeds. Indian Jour. Med. Res. 32:131.

Boucher, R. V.: 1944. Efficacy of vitamin D from different sources for turkeys. Jour. Nutr. 27 403.

Briggs, G. M., Jr: 1946. Nicotinic acid deficiency in turkey poults and the occurrence of perosis Jour. Nutr. 31:79.

———, Groschke, A. C., and Lillie, R. J.: 1946. Effects of proteins low in tryptophane on growth of chickens and on laying hens receiving nicotinic acid low rations. Jour. Nutr. 32.659.

———, Mills, R. C., Elvehjem, C. A., and Hart, E. B.: 1942a. Nicotinic acid in chick nutrition. Proc. Soc. Exper. Biol. and Med. 51:59.

———, Mills, R. C., Hegsted, D. M., Elvehjem, C. A., and Hart, E. B.: 1942b. The vitamin B6 requirement of the chick. Poultry Sci. 21:379.

Buckner, G. D., Insko, W. M., Jr., Henry, Amanda H., and Wachs, Elizabeth F.: 1951. Influence of vitamin D on the growth of New Hampshire cockerels, their combs, wattles, gonads, uropygeal glands. Poultry Sci. 30:267.

Carrick, G. W., and Hauge, S. M.: 1925. Presence of the antiscorbutic substance in the livers of chickens fed on scorbutic diets. Jour. Biol. Chem. 63:115.

Chin, D., Anderson, J. B., Miller, R. F., Norris, L. C., and Henser, G. F.: 1958. The vitamin B_{12} requirement of White Leghorn hens. Poultry Sci. 37:335.

Couch, J. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G.: 1948. Relation of biotin to congenital deformities in the chick. Anat. Record 100:29.

Cravens, W. W., McGibbon, W. H., and Sebesta, E. E.: 1944. Effect of biotin deficiency on embryonic development in the domestic fowl. Anat. Record 90:55.

———, Sebesta, E. E., Halpin, J. G., and Hart, E. B.: 1942. Effect of biotin on reproduction in the domestic fowl. Proc. Soc. Exper. Biol. and Med. 50:101.

———, Sebesta, E. E., Halpin, J. G., and Hart, E. B.: 1943. Effect of vitamin B_2 on egg production and hatchability. Poultry Sci. 22:94.

———, Sebesta, E. E., Halpin, J. G., and Hart, E. B.: 1946. Studies on the pyridoxine requirements of laying and breeding hens. Poultry Sci. 25:80.

———, and Snell, E. E.: 1950. Reversal of aminopterin inhibition in the chick embryo with the Leuconostoc citrovorum factor. Proc. Soc. Exper. Biol. and Med. 75:43.

Dam, H., and Glavind, J.: 1939. Alimentary exudative diathesis and its relation to vitamin E. Skand. Arch. Physiol. 82:299.

Daniel, L. J., Farmer, F. A., and Norris, L. C.: 1946. Folic acid and perosis. Jour. Biol. Chem. 163:349.

——— and Norris, L. C.: 1949. Effect of oxythiamin on the growth of chicks. Proc. Soc. Exper. Biol. and Med. 72:165.

Davies, A. W.: 1952. Lowered liver vitamin A reserves in avian coccidiosis. Nature 170:849.

Davis, H. J., Norris, L. C., and Heuser, G. F.: 1938a. The role of vitamin G in reproduction in poultry. Poultry Sci. 17:81.

———, Norris, L. C., and Heuser, G. F.: 1938b. Further evidence on the amount of vitamin G required for reproduction in poultry. Poultry Sci. 17:87.

Doyle, L. P.: 1929. Rickets in mature chickens. Poultry Sci. 4:146.

Elvehjem, C. A., and Neu, V. F.: 1932. Studies in vitamin A avitaminosis in the chick. Jour. Biol. Chem. 97:71.

Engel, R. W., Phillips, P. H., and Halpin, J. G.: 1940. The effect of a riboflavin deficiency in the hen upon embryonic development of the chick. Poultry Sci. 19:135.

Erasmus, J., Scott, M. L., and Levine, P. P.: 1960. A relationship between coccidiosis and vitamin A nutrition in chickens. Poultry Sci. 39:565.

Fell, H. B., and Mellanby, E.: 1953. Metaplasia produced in cultures of chick ectoderm by high vitamin A. Jour. Physiol. 119:470.

Fisher, H., and Hudson, C. B.: 1956. Chick viability and pantothenic acid deficiency in the breeding diet — a case report. Poultry Sci. 35:487. (Research note.)

Fritz, J. C., Archer, W. F., and Barker, D. K.: 1942. Observations on the stability of vitamin D. Poultry Sci. 21:361.

———, Hooper, J. H., and Moore, H. P.: 1945. Calcification in the poult. Poultry Sci. 24:524.

Frölich, A.: 1954. Relation between vitamin D and vitamin B_{12}. Nature 174:462.

Frost, D. V., Dann, F. P., and McIntire, F. C.: 1946. Adequacy of the known synthetic vitamins for normal feathering and pigmentation in chicks. Proc. Soc. Exper. Biol. and Med. 61:65.

Fuller, H. L., and Dunahoo, W. S.: 1959. The effect of various drug additives on the vitamin B_{12} requirement of chicks. Poultry Sci. 38:1150.

———, Field, R. C., Roncalli-Amici, R., Dunahoo, W. S., and Edwards, H. M., Jr.: 1961. The vitamin B_{12} requirement of breeder hens. Poultry Sci. 40:249.

———, and Kifer, P. E.: 1959. The vitamin B_{12} requirement of chicks. Poultry Sci. 38:255.

Ganguly, J., Mehl, J. W., and Deuel, H. J., jr.: 1953. Studies on carotenoid metabolism. XII. The effect of dietary carotenoids on the carotenoid distribution in the tissues of chickens. Jour. Nutr. 50:59.

Gillis, M. B., Heuser, G. F., and Norris, L. C.: 1948. Pantothenic acid in the nutrition of the hen. Jour. Nutr. 35:351.

Glazener, E. W., Mattingly, J. P., and Briggs, G. M.: 1946. Abnormal blackening of the feathers of New Hampshire chicks as the result of vitamin D deficiency. Poultry Sci. 25:85. (Research note.)

Glover, J., and Redfearn, E. R.: 1954. The mechanism of the transformation of β carotene into vitamin A in vivo. Biochem. Jour. 58(2):xv.

Goldstein, J., and Scott, M. L.: 1956. An electrophoretic study of exudative diathesis in chicks. Jour. Nutr. 60:349.

Green, R. G., Carlson, W. E., and Evans, C. A.: 1942. The inactivation of vitamin B_1 in diets containing whole fish. Jour. Nutr. 23:165.

Griminger, P.: 1957. On the vitamin K requirement of turkey poults. Poultry Sci. 36:1227.

Gutteridge, H. S., and Novikoff, M.: 1947. The effect of natural and synthetic vitamins D_2 and D_3 and of thyroprotein on egg shell quality. Poultry Sci. 26:210. (Research note.)

Hall, G. E., and King, E. J.: 1951. Calcium-phosphorus metabolism in the chicken. 1. The effect of irradiated ergosterol (vitamin D). Poultry Sci. 10:132.

Haque, M. E., Lillie, R. J., Shaffner, C. S., and Briggs, G. M.: 1949. Response of vitamin deficient chicks to the sex hormones. Poultry Sci. 28:914.

Hart, E. B., Steenbock, H., Lepkovsky, S., and Halpin, J. G.: 1925. The nutritional requirements of baby chicks. 111. The relation of light to the growth of the chicken. Jour. Biol. Chem 58:33.

———. Steenbock, H., Lepkovsky, S., and Halpin, J. G.: 1925b. The nutritional requirement of the chicken. VI. Does the chicken require vitamin C? Jour. Biol. Chem. 66.813.

———. Steenbock, H., Lepkovsky, S., Kletzem, S. W. F., Halpin, J. G., and Johnson, O. N.: 1925a. The nutritional requirement of the chicken. V. The influence of ultra-violet light on the production, hatchability, and fertility of the egg. Jour. Biol. Chem. 65.579.

Henry, Kathleen M., and Kon, S. K.: 1946. Vitamin B_{12} and protein metabolism. Brit. Jour. Nutr. 10:39.

Hertz, R.: 1945. The quantitative relationship between stilbestrol response and dietary "folic acid" in the chick. Endocrinology 37:1.

Heuser, G. F.: 1935. A preliminary report on the vitamin G requirement of turkeys. Poultry Sci. 14:376.

———, and Norris, L. C.: 1929. Rickets in chicks. 111. The effectiveness of midsummer sunshine and irradiation from a quartz mercury vapor arc in preventing rickets in chicks. Poultry Sci. 8:89.

——— and Scott, M. L.: 1953. Studies in duck nutrition. V. Bowed legs in ducks, a nutritional disorder. Poultry Sci. 32:137.

Hill, C. H., and Scott, M. L.: 1951. Studies on the role of cysteine in the activation of folic acid conjugase. Jour. Biol. Chem. 189.651.

——— and Scott, M. L.: 1952a. Studies on the enzymatic release of citrovorum factor. Jour. Biol. Chem. 196:189.

———, and Scott, M. L.: 1952b. The effect of ascorbic acid on the citrovorum factor-liberating enzyme of chick liver. Jour. Biol. Chem. 196:195.

Hill, F. W.: 1954. Unpublished results.

———, Norris, L. C., and Scott, M. L.: 1954. The riboflavin requirement of Single Comb White Leghorns for egg production and reproduction. Proc. 1954 Cornell Nutr. Conf., p. 42.

———, Scott, M. L., Norris, L. C., and Heuser, G. F.: 1961. Reinvestigation of the vitamin A requirements of laying and breeding hens and their progeny. Poultry Sci. 40:1245.

Hinshaw, W. R., and Lloyd, W. E.: 1934. Vitamin A deficiency in turkeys. Hilgardia 8.281.

Hogan, A. G., Richardson, L. R., Patrick, H., O'Dell, B. L., and Kempster, H. L.: 1941. Vitamin B_1 and chick nutrition. Poultry Sci. 20:180.

Hooper, J. H., Halpin, J. L., and Fritz, J. C.: 1942. The feeding of single massive doses of vitamin D to birds. Poultry Sci. 21:472. (Abst.)

Hou, H. C.: 1928. Studies on the glandula uropygialis of birds. Chinese Jour. Physiol. 2:345.

———: 1931. Relation of preen glands of birds to rickets. Chinese Jour. Physiol. 5:11.

Hughes, J. S., and Payne, L. F.: 1924. Influence of ultra-violet light on young laying hens. Science 60:549.

Jukes, T. H., and Bird, F. H.: 1942. Prevention of perosis by biotin. Proc. Soc. Exper. Biol. and Med. 49.231.

Jungherr, E.: 1943 Nasal histopathology and liver storage in subtotal vitamin A deficiency of chickens. Storrs (Conn.) Agr. Exper. Sta. Bull. 250.

Kahn, R. H.: 1954. Effect of estrogen and of vitamin A on vaginal cornification in tissue culture. Nature 174:317.

Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P.: 1949. Effect of folic acid, "4 amino" folic acids and related substances on growth of chick embryo. Proc. Soc. Exper. Biol. and Med. 71:442.

Keane, K. W., Collins, R. A., and Gillis, M. B.: 1956. Isotopic tracer studies on the effect of vitamin D on calcium metabolism in the chick. Poultry Sci. 35:1216.

Kline, Irene T.: 1955. Relationship of vitamin B_{12} to stilbestrol stimulation of the chick oviduct. Endocrinology 57:120.

———, and Dorfman, R. I.: 1951. Citrovorum factor and oviduct response to stilbestrol in aminopterin-treated chicks. Proc. Soc. Exper. Biol. and Med. 76:205.

Koch, E. M., and Koch, F. C.: 1941. The provitamin D of the covering tissues of chickens. Poultry Sci. 20.33.

Krause, D. J.: 1922. The water content of the tissues in experimental beriberi. Am. Jour. Physiol. 60:234.

Landauer, W.: 1954. The effect of estradiol benzoate and corn oil on bone structure of growing cockerels exposed to vitamin D deficiency. Endocrinology 55:686.

Lepkovsky, S., and Jukes, T. H.: 1936. The response of rats, chicks, and turkey poults to crystalline vitamin G (flavin). Jour. Nutr. 12.515.

———, Taylor, L. W., Jukes, T. H., and Almquist, H. J.: 1938. The effect of riboflavin and the filtrate factor on egg production and hatchability. Hilgardia 11.559.

Lillie, R. J., and Bird, H. R.: 1949. A breed difference in feather pigmentation of vitamin D-deficient chicks. Poultry Sci. 28:160. (Research note)

——, Olsen, M. W., and Bird, H. R.: 1949. Role of vitamin B_{12} in reproduction of poultry. Proc. Soc. Exper. Biol. and Med. 72:598.

Little, P. A., Sampath, A., Paganelli, V., Locke, A., and SubbaRow, Y.: 1948. The effect of folic acid and its antagonists on Rous chicken sarcoma. Trans. N. Y. Acad. Sci. 10:91.

Lucas, H. L., Heuser, G. F., and Norris, L. C.: 1946. The unexpected high requirements of chicks for pyridoxine. Poultry Sci. 25:137.

Ludwig, K. S.: 1953. Vitamin A-mangel und überdosierung und ihre Beziehungen zum Gehalt an alkalischer phosphatase der epiphysenfuge. Intern. Zeit für Vitaminforschung 25.98.

McCarrison, R.: 1918. The pathogenesis of deficiency disease. Indian Jour. Med. Res. 6.275.

McChesney, E. W.: 1943. The comparative effect of vitamins D_2 and D_3 and dihydrotachysterol given orally and intramuscularly. Jour. Nutr. 26:81.

McElroy, L. W., and Jukes, T. H.: 1940. The formation of the anti-egg-white injury factor (biotin) in the rumen of the cow. Proc. Soc. Exper. Biol. and Med. 45:296.

McGinnis, J., and Carver, J. S.: 1947. The effect of riboflavin and biotin in the prevention of dermatitis and perosis in turkey poults. Poultry Sci. 26:564.

——, Hsu, P. T., and Graham, W. D.: 1948. Studies on an unidentified factor required by chicks for growth and protein utilization. Poultry Sci. 27:674.

——, Kosin, I. L., and Decker, Annabelle: 1947. The influence of vitamin D, diethylstilbestrol, thiouracil and iodinated casein on feather pigmentation in New Hampshire chicks. Poultry Sci. 26:550. (Abst.)

——, Norris, L. C., and Heuser, G. F.: 1942. An unidentified nutritional factor required by the chick for feather pigmentation. Jour. Biol. Chem. 145:341.

Milby, T. T., and Thompson, R. B.: 1943. The stability of vitamin D in D-activated animal sterol when fed to turkey poults. Poultry Sci. 22:357.

Miller, M. W., Joukovsky, V., and Hokanstad, N.: 1942. The effect of manganese sulfate on the stability of vitamins A and D of cod liver oil when stored in mixed feeds. Poultry Sci. 21:200

Milne, H. I.: 1936. Studies of perosis in chicks. Proc. Sixth World's Poultry Cong. 2:155.

Morrison, A. B., and Norris, L. C.: 1956 Failure of thioctic acid to stimulate chick growth. Poultry Sci. 35:739.

Motzok, I., Hill, D. C., and Branion, H. D.: 1946. Antirachitic efficacy of dihydrotachysterol for chicks. Poultry Sci. 25:644. (Research note.)

Mussehl, F. E., and Bancroft, P. M.: 1925. Nutrient requirements of growing chicks. Poultry Sci. 4:118.

National) Academy of Sciences-National Research Council: 1960. Nutrient requirements for poultry. Publ. 827.

Norris, L. C., Heuser, G. F., and Wilgus, H. S., Jr.: 1929 Effect of storage in finely divided feeds upon the stability of the D vitamin of cod liver oil. Cornell Univ. Agr. Exper. Sta. Mem. 126

——, Heuser, G. F., and Wilgus, H. S., Jr.: 1930. Is the chief value of milk for feeding poultry due to the presence of a new vitamin? Poultry Sci. 9:153.

——, and Ringrose, A. T.: 1930. The occurrence of a pellagrous-like syndrome in chicks. Science 71:643.

Olcese, O., Couch, J. R., Quisenberry, J. H., and Pearson, P. B.: 1950. Congenital anomalies in the chick due to vitamin B_{12} deficiency. Jour. Nutr. 41:423.

Pappenheimer, A. M., and Goettsch, M.: 1931. A cerebellar disorder in chicks, apparently of nutritional origin. Jour. Exper. Med. 53:11.

——, Goettsch, M., and Jungherr, E.: 1939. Nutritional encephalomalacia in chicks and certain related disorders of domestic birds. Storrs (Conn.) Agr. Exper. Sta. Bul. 229.

——, and Graft, S.: 1932. Blood volume on normal chicks and in chicks with nutritional encephalomalacia. Proc. Soc. Exper. Biol. and Med. 30:321.

Patterson, E. L., Milstrey, R., and Stokstad, E. L. R.: 1957. Effect of selenium in preventing exudative diathesis in chicks. Proc. Soc. Exper. Biol. and Med. 95:617.

Peeler, H. T., Miller, R. F., Carbon, C. W., Norris, L. C., and Heuser, G. F.: 1951. Studies of the effect of vitamin B_{12} on hatchability. Poultry Sci. 30:11.

Phillips, P. H., and Engel, R. W.: 1938. The histopathology of neuromalacia and "curled toe" paralysis in the chick fed low riboflavin diets. Jour. Nutr. 16:451.

——, and Engel, R. W.: 1939. Some histopathologic observations on chicks deficient in the chick antidermatitis factor or pantothenic acid. Jour. Nutr. 18:227.

Polk, H. D., and Sipe, G. R.: 1940. The effect of vitamin A deficiency on malposition of the chick embryo. Poultry Sci. 19.396.

Quackenbush, F. W., Cox, R. P., and Steenbock, H.: 1942. Tocopherol and the stability of carotene. Jour. Biol. Chem. 145:169.

Richardson, L. R., Hogan, A. G., and Miller, O. N.: 1942. The relation of biotin to perosis in chicks. Mo. Agr. Exper. Sta. Res. Bul. 345.

Ringrose, A. T., Norris, L. C., and Heuser, G. F.: 1931. The occurrence of a pellagra-like syndrome in chicks. Poultry Sci. 10:166.

Robblee, A. R., and Clandinin, D. R.: 1950. The use of B vitamins in practical turkey starters. Poultry Sci. 29:777.

Robertson, E. I., Angstrom, C. I., Clark, H. C., and Shinam, M.: 1949. Field research on "stunted chick" disease. Poultry Sci. 28:14.

———, Daniel, L. J., Farmer, F. A., Norris, L. C., and Heuser, G. F.: 1946. The folic acid requirements of chicks for growth, feathering, and hemoglobin formation. Proc. Soc. Exper. Biol. and Med. 62:97.

———, Fiala, Grace F., Scott, M. L., Norris, L. C., and Heuser, G. F.: 1947. Response of anemic chicks to pteroylglutamic acid. Proc. Soc. Exper. Biol. and Med. 64:441.

Salsbury, R. M., Edmondson, J., Poole, W. S. H., Bobby, F. C., and Birnie, H.: 1962. Exudative diathesis and white muscle disease of poultry in New Zealand. Proc. Twelfth World's Poultry Cong. Sydney, Australia, p. 579.

Schneider, H. A., Hachian, H., and Steenbock, H.: 1939. The anti-acrodynic properties of certain foods. Jour. Nutr. 18:99.

Schwarz, K., Bieri, J. G., Briggs, G. M., and Scott, M. L.: 1957. Prevention of exudative diathesis in chicks by Factor 3 and selenium. Proc. Soc. Exper. Biol. and Med. 95:621.

Scott, M. L.: 1940. Studies on the enlarged hock disorder (perosis) in turkeys. Jour. Nutr, 40.611.

———: 1951. Studies on the enlarged hock disorder in turkeys. 3. Evidence of the detrimental effect of fish liver oil and the beneficial effect of dried brewers' yeast and other materials. Poultry Sci. 30:846.

———: 1953. Prevention of the enlarged hock disorder in turkeys with niacin and vitamin E. Poultry Sci. 32.670.

———: 1962a. Antioxidants, selenium and sulfur amino acids in the vitamin E nutrition of chicks. Nutr. Abst. and Revs. 32:1.

———: 1962b. Vitamin E in health and disease of poultry. Vitamins and Hormones 20.621.

———, Bieri, J. G., Briggs, G. M., and Schwarz, K.: 1957. Prevention of exudative diathesis by Factor 3 in chicks on vitamin E-deficient Torula yeast diets. Poultry Sci. 36:1155. (Abst.)

———, and Heuser, G. F.: 1952. Studies in duck nutrition. 4. Bowed legs in ducks caused by niacin deficiency. Poultry Sci. 31:752. (Research note.)

———, Hill, F. W., Norris, L. C., Dobson, D. C., and Nelson, T. S.: 1955. Studies on vitamin E in poultry nutrition. Jour. Nutr. 56:387.

———, and Stoewsand, G. S.: 1961. A study of ataxias of vitamin A and vitamin E deficiencies in the chick. Poultry Sci. 40:1517.

Seifried, O.: 1930a. Studies on A-avitaminosis in chickens. II. Lesions of the upper alimentary tract and their relation to some infectious diseases. Jour. Exper. Med. 52:533.

———: 1930b. Studies on A-avitaminosis in chickens. I. Lesions of the respiratory tract and their relation to some infectious diseases. Jour, Exper. Med. 52:519.

———, and Heidegger, E.: 1933. Vitamin-D-Schaden beim Huhn. Tierärtzl. Rundschau 39:171.

Sherwood, R. M.: 1939. Vitamin A requirements of poultry. Proc. Seventh World's Poultry Cong., p. 123.

———, and Fraps, G. S.: 1932. The quantities of vitamin A required by pullets for maintenance and for egg production. Tex. Agr. Exper. Sta. Bul. 468.

———, and Fraps, G. S.: 1940. Unpublished data.

Singsen, E. P., Bunnell, R. H., Kozeff, A., Matterson, L. D., and Jungherr, E. L.: 1953. Studies on encephalomalacia in the chick. 2. The protective action of diphenyl p-phenylenediamine against encephalomalacia. Poultry Sci. 32:924. (Abst.)

———, and Mitchell, H. H.: 1915. Phosphorus in poultry nutrition. 1. The relation between phytin and different sources of vitamin D. Poultry Sci. 24:479. (Research note.)

Slinger, S. J., Pepper, W. F., and Motzok, I.: 1954. Interrelationship between vitamin E and phosphorus in preventing perosis in turkeys. Jour. Nutr. 52:355.

Somogyi, J. C.: 1952. Die antianeurin Factoren. Hans Huber, Bern, Switzerland.

Steenbock, H., and Bellin, S. A.: 1953. Vitamin D and tissue citrate. Jour. Biol. Chem. 205:985.

———, and Herting, D. C.: 1955. Vitamin D and growth. Jour. Nutr. 57:449.

Stoewsand, G. S., and Scott, M. L.: 1961. The vitamin A requirements of breeding turkeys and their progeny. Poultry Sci. 40:1255.

Stokstad, E. L. R., Hoquind, H. P., and Patterson, E. L.: 1953. Role of pteroylglutamic acid in animal nutrition. Fed. Proc. 12:430.

Sumner, J. B., and Dounce, A. L.: 1939. Carotene oxidase. Enzymologia 7:130.

Sunde, M. L., Cravens, W. W., Bruins, H. W., Elvehjem, C. A., and Halpin, J. G.: 1950a. The pteroylglutamic acid requirement of laying and breeding hens. Poultry Sci. 29 220.

———, Cravens, W. W., Elvehjem, C. A., and Halpin, J. G.: 1950b. The effect of folic acid on embryonic development of the domestic fowl. Poultry Sci. 29 696.

Supplee, W. C., Combs, G. F., and Romoser, G. L.: 1956. Failure to obtain growth responses with thiotic acid in chicks from different sources. Arch. Biochem. and Biophys. 61:140.

van Niekerk, J., and Franken, F.: 1937. Over den invloed van zeer groote hoeveelheden antirachitisch vitamine van denkplken oosprong bij kuikens. Landhoukwundig Tijdschr. 49:742.

Vedder, E. B., and Clark, E.: 1912. A study of polyneuritis gallinarum. A fifth contribution to the etiology of beriberi. Philippine Jour. Sci., Sect. B, 7:423.

Walter, E. D., and Jensen, L. S.: 1963. Effectiveness of selenium and noneffectiveness of sulfur amino acids in preventing muscular dystrophy in the turkey poult. Jour. Nutr. 80.327.

Wolbach, S. B., and Hegsted, D. M.: 1952. Vitamin A deficiency in the duck. Skeletal growth and central nervous system. Arch. Path. 54:548.

————, and Hegsted, D. M.: 1953. Hypervitaminosis A in young ducks. The epiphyseal cartilages. Arch. Path. 55:47.

Wolf, A., and Pappenheimer, A. M.: 1931. The histopathology of nutritional encephalomalacia of chicks. Jour. Exper. Med. 54:399.

Wolf, G., and Johnson, B. C.: 1960. Vitamin A and mucopolysaccharide biosynthesis. Vitamins and Hormones 18:439.

Woollam, D. H. M., and Millen, J. W.: 1955. Effect of vitamin A deficiency on the cerebrospinal fluid pressure of the chick. Nature 175:41.

Yacowitz, H., Norris, L. C., and Heuser, G. F.: 1951. Evidence for an interrelationship between vitamin B_{12} and pantothenic acid. Jour. Biol. Chem. 192:141.

Young, R. J., Norris, L. C., and Heuser, G. F.: 1955. The chick's requirement for folic acid in the utilization of choline and its precursors betaine and methylaminoethanol. Jour. Nutr. 55:353.

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8

Pullorum Disease

In 1899 the etiological agent of pullorum disease was discovered by Rettger (1900). He first described the disease as a "Fatal Septicemia of Young Chicks," but later (1909) he designated it as "White Diarrhea." However, in that same year, in a subsequent report, Rettger and Stoneburn (1909) applied the term "Bacillary White Diarrhea" in order to distinguish it from other avian diseases which might be classified under a common terminology of "White Diarrhea" as was reported by Jones (1911).

In 1929 at the Second Annual Meeting of Investigators and Workers in Bacillary White Diarrhea Control, Rettger and Plastridge (1932), at the suggestion received from a research member of the Pennsylvania Department of Agriculture, proposed that the term "Pullorum Disease" be substituted for "Bacillary White Diarrhea." This new terminology was internationally adopted because of its brevity, specificity, and appropriateness in designating a disease entity which af-

fected not only chicks but also mature poultry and fowl other than chickens.

HISTORY

The isolation of the causative agent of pullorum disease by Rettger (1900) in 1899 gave an insight into the serious chick-raising problem reported in the lay press (E. A. H., 1905; Gifford, 1905; Graham, 1904) which appeared before and after the epoch-making discovery. At the close of the nineteenth century, this malady was considered a very serious menace to the poultry industry. During the first decade of the twentieth century, investigators definitely proved that pullorum disease was an egg-borne infection. The cycle of infection involved an infected hen laying infective eggs, hatching infected chicks, which could develop into mature infected stock.

During the second decade, Jones (1913b), and later others (Gage et al., 1914; Rettger et al., 1914), announced the practical application of the macroscopic

tube agglutination test for the detection of "carriers" of the organism. The application of this test in the control and eradication of the disease was carried out rather extensively in some of the eastern states so that toward the close of the second decade, official state testing programs were inaugurated.

The progressive development and expansion of the baby chick industry through more modern methods of incubation, brooding, and transportation have influenced the dissemination of the disease. Incubator transmission has been, and still is, an important factor in the spread of pullorum disease from plant to plant by means of flock replacements.

Another event which has influenced the pullorum status throughout the world was the organization of the Conference of Investigators and Workers in Bacillary White Diarrhea Control (W. R. H., 1928), composed first of representatives from the New England states and later (Anon., 1930) enlarged to include other eastern states and provinces in Canada. This conference has made a concerted effort to bring about standardization and uniformity of methods and to stimulate an interest in the practical eradication of the disease from breeding flocks.

The Conference of Research Workers in Animal Diseases of North America (Anon., 1933) formulated "Standard Methods of Diagnosis of Pullorum Disease in Barnyard Fowl" which were adopted by this organization and also by the United States Livestock Association in 1932. These methods of diagnosis have served as valuable guides in combating pullorum disease.

Schaffer et al. (1931) announced the development of the modified whole-blood method in which stained antigen is employed. In view of its apparent simplicity, it has been widely used with the result that many infected birds have been detected, and thus their removal from breeding flocks was made possible.

The United States Department of Agriculture inaugurated the National Poultry Improvement Plan which represents a national effort to control and eradicate pullorum disease in poultry flocks (National Poultry Improvement Plan, 1941).

The above-mentioned events point out that during the past years the poultry industry has been made more secure through the publication of these scientific facts and through the adoption of official federal and state plans which are being employed to combat pullorum disease.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Pullorum infection is world-wide in its distribution. It is likely to be found wherever poultry is being raised. Prior to the date the organism was discovered by Rettger (1900), the disease had been observed in the United States and Canada by those engaged in poultry husbandry. Later reports (Reis and Nobrega, 1936) revealed the recognition of the disease in England, different parts of continental Africa, Asia, Australia, Europe, Japan, Korea, and South America.

Economic losses of serious proportions were reported during the last decade in the nineteenth century. As the poultry industry developed, especially in the breeding and hatching phases, the incidence of the disease was permitted to become greater, and the infection was disseminated more widely throughout the United States and Canada. During a later period this likewise was true in other countries.

Losses from the disease may be experienced through severe chick mortality, reduced fertility and hatchability, retardation in growth, reduced egg production, increased mortality among adult stock, and a reduction in the sales quality of the stock.

Rettger (1900, 1901) in his early observations of the disease found that among hen-reared and among artificially brooded chicks, total mortalities might approximate 85 per cent during the first four weeks of age. Later reports (Jones, 1911; Kaupp, 1917) reveal similar or even higher
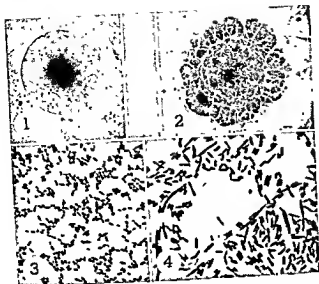
FIG. 8.1 — (1) Isolated colony on primary culture. Meat extract agar. 40 hours old. ×15. (2) Isolated colony on primary culture. Liver infusion agar, 40 hours old. ×15. (3) Cells in a smear prepared from colony illustrated in 1. ×1,200. (4) Cells in a smear prepared from colony illustrated in 2. ×1,200.

be profitable and that variations in production may be expected depending upon the localization and degree of infection. It is generally accepted that pullorum infection may impair production and should for that reason, as well as for other reasons, not be tolerated in a flock.

Through the inauguration of official control programs and the establishment of official grades for pullorum-tested flocks, it has been made possible to identify flocks as to their official pullorum disease status and thereby afford the buying public an opportunity to buy stock of the highest quality. An abundance of pullorum-free stock is available at the present time in most areas and should be adequate to meet the demands of the buying public.

ETIOLOGY

Pullorum disease is a bacterial infection caused by an organism which Rettger (1900, 1909) first designated as a bacillus and a few years later named *Bacterium pullorum*.

More recently the systematic bacteriologist has classified the etiological agent in the "Salmonella" genus, and at the present time, the organism is recognized as *Salmonella pullorum*. It has many features in common with other members of the Salmonella group.

The organism is a long, slender rod (.3–.5 × 1–2.5μ) with slightly rounded ends (Fig. 8.1–3). It readily stains with ordinary basic anilin dyes and is Gram-negative. The cells occur singly, with chains of more than two bacilli being rarely found. An occasional filament and large cell may be found in smear preparations. It is nonmotile, nonliquefying, nonchromogenic, nonsporogenic, and facultatively anaerobic. Optimum growth occurs at 37° C. and under normal atmospheric conditions. On meat extract agar (pH 7.0–7.2) heavily seeded with inoculum, the colonies appear discrete, smooth, glistening, homogeneous, entire, dome-shaped, transparent, and varying in form from round to angular (Fig. 8.1–1). On chicken infusion agar, the growth is slightly more luxuriant, with colonies possessing a lesser degree of transparency. On liver infusion agar, the growth is even more luxuriant and markedly translucent (Fig. 8.1–2 and 4). Congested colonies remain small (1 mm. or less), but isolated colonies may attain a diameter of 3 to 4 mm. or more. Surface markings may appear as the colony increases in size and age, but as a rule the young colony on a heavily seeded plate changes little with age.

The following substances are attacked

with acid and with or without gas production: arabinose, dextrose, galactose, levulose, mannite, mannose, rhamnose, and xylose. Substances not attacked include adonite, dextrin, dulcitol, erythrol, glycerol, inositol, inulin, lactose, raffinose, saccharose, salicin, sorbite, and starch. Maltose is attacked very infrequently as has been reported by Edwards (1928), Hendrickson (1927), Hinshaw (1941), and Pacheco and Rodrigues (1936). However, the results in some instances were attributed to the materials and methods employed for the cultivation of the organism. Edwards (1928) concluded that acid production in maltose by *S. pullorum* was made possible through the hydrolization of the sugar by the alkali that slowly developed upon prolonged incubation. Hendrickson (1927) observed that when serum water was used for sugar base, maltose was fermented by *S. pullorum*. Pacheco and Rodrigues (1936) encountered similar findings and claimed the acid production by the organism was the result of serum-enzyme hydrolysis of the sugar. Van Roekel (1935) reported a laboratory strain which had been considered as a maltose nonfermenting organism, but after a lapse of several years since its original isolation it acquired and retained the ability to attack maltose. No plausible explanation could be given for the sudden change in the maltose-fermenting characteristic. Subsequent investigations (Van Roekel *et al.*, 1937) revealed that strains which possessed a potential tendency to ferment maltose could be identified by cultivating them in a maltose-peptone solution for a period of time Strains undergoing a change in behavior toward maltose would exhibit red and white colonies when plated on a modified Endo's medium (maltose substituted for lactose). Strains that produced both maltose-fermenting and nonmaltose-fermenting colonies exhibited only nonmaltose-fermenting colonies after being subjected to animal passage. An apparently pure maltose-fermenting strain did not lose this property when subjected to animal passage. It is apparent that *S. pul-*

lorum may display variation in its behavior in the fermentation of maltose, and for that reason this sugar cannot be regarded of value in the identification of the organism. Variation in the behavior of some strains may be observed occasionally, especially in regard to gas production. Litmus milk remains practically unchanged. Indol and acetylmethyl carbinol are not formed. Hydrogen sulfide is produced, and nitrates are reduced.

The organism can be cultivated on special media such as dextrose-lactose agar (Mallmann and Snyder, 1929), brilliant green agar (Mallmann, 1929), Endo's agar, cysteine gelatin (Hinshaw, 1941), and sodium tartrate and mucate media (Mallmann, 1931b) which may be of value in the differentiation from other organisms. Bushnell and Porter (1945) tested several types of media for the cultivation and isolation of *S. pullorum*. They concluded that no single medium used proved satisfactory for isolation of *S. pullorum*. In the selection of the medium for the isolation of *S. pullorum* consideration must be given to the source of material to be examined. In isolating *S. pullorum* from the intestine, desoxycholate citrate, bismuth sulfite, and S-S agar were found to be the most satisfactory. Tetrathionate broth was recommended as an enrichment medium.

Growth studies of *S. pullorum* strains in different media revealed that the rate of growth was in a declining order for nutrient broth. one per cent tryptose-water, and a synthetic medium (Schoenhard and Stafseth, 1953). Other workers have demonstrated that the organism is capable of synthesizing glutamic acid and alanine when propagated in synthetic media containing threonine (Jones and Holtman, 1953). Also the virulence of the organism was maintained as readily in the synthetic medium as by animal passage (Gilfillan *et al.*, 1955). The rate of growth of *S. pullorum* could be increased markedly by the addition of yeast extract to trypticase soy agar (Stokes and Bayne, 1957). The authors suggest that the addition of yeast

extract to selective agars may be helpful in the isolation of slow-growth Salmonella.

Reference to the literature (Hinshaw, 1941; Pacheco and Rodrigues, 1936; and Rettger and Plastridge, 1932) reveals that a disagreement concerning the results of the biochemical behavior of *S. pullorum* is to be found; especially is this true among European and American workers. The former for the most part are inclined to regard *S. pullorum* and *S. gallinarum* as identical species. This is difficult to comprehend when certain areas in the United States are observed to be relatively free of fowl typhoid as based on field and laboratory observations, whereas pullorum disease is more prevalent. This statement is based on diagnoses obtained by the standard criteria employed in differentiating these two diseases.

While *S. pullorum* is generally accepted as being a stable distinct species, Plastridge and Rettger (1930, 1932), Mallmann (1932), and Van Roekel (1935) have observed that the organism may vary markedly in many of its characteristics (Fig. 8.2–5 to 8). The toxicogenic properties of *S. pullorum* were investigated by Hanks and Rettger (1932), who observed that *S. pullorum* cells contained an extractable heat-resistant poison which is highly toxic for rabbits and is capable of killing guinea pigs and mice. Chicks revealed no noticeable symptoms of illness, regardless of the route by which the material was introduced. They concluded that pullorum disease appears to be a septicemia rather than a toxemia.

The antigenic composition of *S. pullorum*, according to the Kauffmann-White schema (Salmonella Subcommittee, 1934), consists only of the O-antigen. Its antigenic structure is similar to that of *S. gallinarum*. However, the O-antigen of *S. pullorum* and *S. gallinarum* has something in common with somatic antigen of other members in the Salmonella genus.

Edwards and Bruner (1946), in their study regarding the antigenic components of *S. pullorum*, conclude the following: "The antigenic formula of *S. pullorum* is

IX, XII₁, [XII₂], XII₃. In normal cultures the XII₂ factor is variable, and forms containing a large amount or a negligible amount of XII₂ can be isolated from the same strain. It is possible for cultures to become fairly well stabilized in either form, thus giving rise to the so-called "standard" strains and "variant" or X strains. The standard strains contain only a small amount of XII₂, but the X strains contain a large amount of the antigen." Wright and Edwards (1948) emphasize that since the differentiation of standard and variant strains is based on a variational phenomenon, a diagnosis must be made with a certain degree of caution and discretion. A cursory examination of a single colony or even several colonies would permit one to conclude that the culture is predominantly in XII₂ or XII₃ form. One should examine 100 colonies or more to determine if the variant strain has become stabilized in the XII₂ form.

The typing results reported by various investigators in this country reveal that approximately 30 per cent of the strains may be of the variant type (Bivins, 1948; Heemstra, 1948; Williams *et al.*, 1949; Rhoades and Alberts, 1950; Snoeyenbos *et al.*, 1952).

Antigenic studies of pullorum and gallinarum cultures isolated in Denmark revealed that the amount of antigenic components IX, XII₂, and XII₃ was identical in 122 strains (Martfedal, 1952).

Marked antigenic stability was observed among three pullorum strains (one standard and two variant) isolated from field cases (Rhoades, 1955), whereas Luzzio *et al.* (1955) reported that the antigenic structure of *S. pullorum* may be influenced quantitatively by environmental factors.

Williams (1953a, 1953b) reported that standard, intermediate, and variant antigenic types could be differentiated with an ammonium sulfate sedimentation test. It was found that a concentration of ammonium sulfate approximating 310 gm. per liter completely cleared the supernatant fluids of standard suspensions, had little or no effect on variant suspensions,
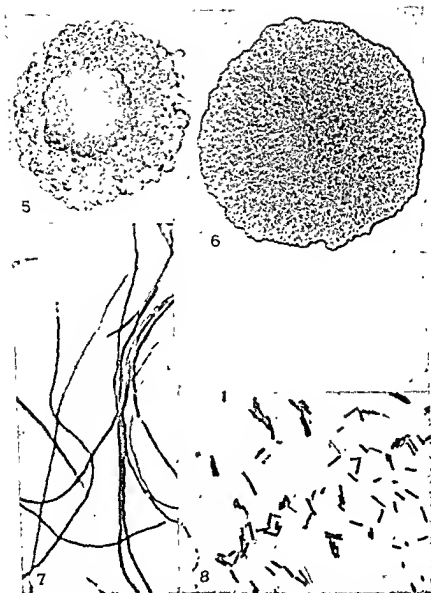
FIG. 8.2 — (5) Colony exhibiting dentated edge, thin periphery which is markedly convoluted, striated, and tenacious. Central portion raised, dense, and faintly convoluted. Three days old. Liver infusion agar. ×10. (6) Colony exhibiting irregular outline, rough surface, opaqueness, and brittleness. Two days old. Meat extract agar. ×25. (7) Filamentous forms in smear prepared from the peripheral portion of the colony illustrated in 5. ×1,200. (8) Cells in a smear prepared from the central portion of the colony illustrated in 5. ×1,200.

and only partially cleared intermediate suspensions. A concentration of the salt approximating 470 gm. per liter was required to clear suspensions of variant and intermediate type strains. Later, Williams and Harris (1956) observed that an ammonium sulfate concentration of 265 gm. per liter completely cleared the supernatant fluids of most *S. gallinarum* suspensions but had considerably less effect on the turbidity of suspensions of standard type cultures of *S. pullorum*. The same authors found that *S. pullorum* and *S gallinarum* antigens lacked hemagglutinating properties (Harris and Williams, 1957).

PATHOGENICITY

Pullorum infection is most prevalent among chickens. However, among the various poultry breeds, a difference in the susceptibility to *S. pullorum* may be apparent. The lighter breeds of chickens, especially the Leghorns, generally speaking, have revealed fewer reactors among tested flocks. Hutt and Scholes (1941) claim the Leghorns possess a greater genetic resistance to the disease. This view is in part also subscribed to by Roberts and Card (1935), although they state that strain differences within the various breeds must be considered. If Leghorns are to be regarded more resistant to pullorum disease on the basis of blood-testing results, then males likewise must be considered more resistant than females. Testing results for a period of years reveal a greater percentage of reactors among females than among males. This difference certainly cannot be attributed entirely to a hereditary trait existing in the male sex because the influence of environmental factors operative in a commercial plant should be recognized. From a series of investigations, Roberts and Card (1935) concluded that heredity is an important factor in resistance and susceptibility to infection with *S. pullorum*. Later Roberts et al. (1939b) reported that resistance and susceptibility of the domestic fowl to pullorum disease are related to the number

of lymphocytes. Resistant chicks revealed a higher lymphocytic count than did the susceptibles. Change in age of the chick also influenced the degree of resistance as well as the lymphocyte number. Scholes and Hutt (1942) claim that high body temperatures and resistance to *S. pullorum* are closely associated. Later Scholes (1942) concluded that resistance to *S. pullorum* more likely depends upon temperature differences than upon differences in the number of lymphocytes in the blood. While the observations reported by Hutt and Scholes (1941), Scholes (1942), Scholes and Hutt (1942), and Roberts et al. (1935, 1939b) command academic interest, from a practical viewpoint the combat against the disease through genetic selection of resistant stock does not appear as effective as the method of eliminating the disease carriers from the breeding flocks. This is substantiated by DeVolt et al. (1941) who state that the development of pullorum-resistant strains is not now a satisfactory substitute for control and eradication programs by the agglutination tests.

Clinical findings reveal that during the course of pullorum infection the levels of arginine, methionine, glycine, and tryptophan are reduced (Ross et al., 1955a). Survival time of chicks infected with the organism was increased following administration of arginine. No increase was noted when arginine was administered 48 hours prior to infection. Glycine and tryptophan had no effect on the survival time of infected chicks whereas methionine had only a slight effect (Ross et al., 1955b). Furthermore, the survival time of pullorum-infected chicks was reduced by the administration of fluoroacetate, malonate, arsenite, citrate, and succinate. Initially, greater concentrations of the organism were found in the organs of fluoroacetate-treated chicks than in the same tissues of the untreated chicks. Varying degrees of impairment of the liver tricarboxylic acid enzymes were observed when assayed during the course of the disease (Gilfillan et al., 1956a, 1956b).

While the chicken appears to be the

natural host of *S. pullorum*, other avian species also have exhibited some degree of susceptibility. Among the barnyard fowl, natural infection has been observed in turkeys (Barboni, 1937; Brunett, 1930; Dalling *et al.*, 1929; Hendrickson and Hilbert, 1930; Hewitt, 1928; and Hinshaw, 1937); ducks (Beaudette, 1938; Chute, 1962; Hinshaw and Hoffman, 1937; Letche, 1929; and Miessner, 1931); and guinea fowl (Bunyea, 1939). Natural outbreaks among pheasants (Hendrickson and Hilbert, 1931; Miessner, 1931; Van Roekel *et al.*, 1947; Williams *et al.*, 1949); quail (Emmel, 1936); sparrows (Dalling *et al.*, 1928; Reis and Nobrega, 1936); European bullfinch (*Pyrrhula europa*) (Hudson and Beaudette, 1929); and pigeons (Reis and Nobrega, 1936; van Heelsbergen, 1929) have also been reported. Canaries, goslings, turtle doves, gold finches, green finches, and bittern are reported vulnerable to infection (Villani, 1937). Edwards (1945) reported a loss of 50 birds among a flock of 75 canaries caused by a natural outbreak of pullorum disease. Thirteen birds were examined and *S. pullorum* was recovered from all of them.

At one time pullorum disease had gained a substantial foothold in commercial turkey flocks (Hinshaw, 1937, 1939). However, in recent years outstanding progress has been made in reducing the disease among turkeys, and a more detailed discussion of the disease among turkeys is presented in the section entitled "Diseases of the Turkey."

Among other fowl which were found to be susceptible either through natural avenues or through artificial means, the disease produced clinical manifestations quite similar to those observed in the chicken and turkey (Dalling *et al.*, 1928; Emmel, 1936; van Heelsbergen, 1929; Van Roekel *et al.*, 1932). Pigeons apparently are quite resistant to the organism, whereas sparrows appear to be very susceptible. The fact that sparrows and pigeons may frequently inhabit poultry plants may offer an explanation why infection appears in previously nonreacting flocks.

Upland game birds such as pheasants and quail exhibit a degree of susceptibility to the extent that game breeders must exercise preventive measures against the disease. Belding (1955) in a field survey isolated *S. pullorum* from 5 of 65 pheasants cultured. It was concluded that a significant number of wild pheasants in Michigan harbor the infection.

Guinea fowl, ducks, and geese appear quite resistant to the organism, but their role in contracting and disseminating the infection should not be considered negligible in the combat against the disease.

Among the mammalian species the rabbit is highly susceptible, as observed by Olney (1928) and Doyle (1925). Guinea pigs, mice, and cats were slightly susceptible, while rats were quite resistant (Mulsow, 1919; Rettger *et al.*, 1916). Benedict *et al.* (1941) recovered the organism from foxes and mink. Edwards and Bruner (1943) isolated a culture of porcine origin. Bruner and Moran (1949) reported the isolation of *S. pullorum* from a heifer, dog, fox, mink, and swine. In a more recent summary extending from January 1, 1957, to July 1, 1961, Moran (1961) reported that *S. pullorum* was isolated from 2 bovine and 3 mink sources. Taylor (1961) in England isolated a culture from a sheep. Human salmonellosis caused by *S. pullorum* has been reported by Edwards and Bruner (1943), Felsenfeld and Young (1944), Mitchell *et al.* (1946), Rose (1962), and Williams (1964). McCullough and Eisele (1951) produced salmonellosis with various strains of *S. pullorum* among inmates in a penal institution. Explosive onset of the illness, high fever, prostration, prompt recovery, and difficulty to recover the organism from stools were noteworthy.

MODES OF TRANSMISSION

The manner in which pullorum infection may be disseminated is of great importance from the standpoint of control, eradication, and prevention of the disease. The etiological agent may be spread through various channels (Fig. 8.3).

Rettger and Stoneburn (1909) isolated
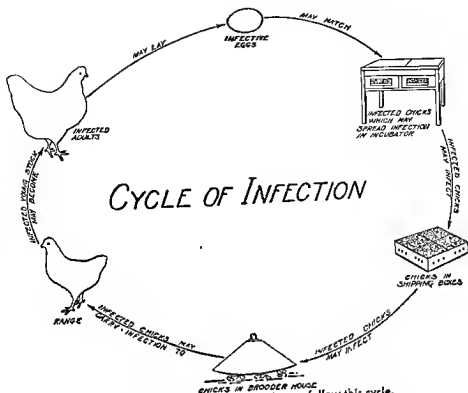
FIG. 8.3 — Pullorum infection in a flock may follow this cycle.

the organism from fresh and incubated eggs which were laid by hens whose progeny succumbed to the disease. Later investigations (Dearstyne et al., 1929; Gage et al., 1914; Jones, 1913a; Rettger and Stoneburn, 1911; Runnells and Van Roekel, 1927a, 1927b; Tittsler et al., 1928) revealed that S. pullorum could be readily recovered from eggs. Runnells and Van Roekel (1927b) reported 33.7 per cent of the eggs laid by reacting hens to be infective. Jones (1913a) and Mathews (1927) reported outbreaks in adult fowls due to S. pullorum as the result of feeding incubated eggs. Van Heelsbergen (1929) emphasized that an important channel of pullorum disease dissemination is through so-called egg-picking. Rettger (1916) stated that eggs harboring large numbers of the organism produce abnormal conditions when fed to young chicks, adult fowls, young rabbits, guinea pigs, and kittens. Olney (1928) encountered a severe outbreak of

the disease among adult rabbits as a result of feeding incubated, infertile eggs. Van Roekel et al. (1932) reported that fresh eggs laid by reacting hens may produce pullorum disease when fed to noninfected hens and pullets, and it appeared that younger birds may contract the disease more readily through eating infective eggs than do older birds. They concluded that the habit of "egg-eating" or "egg-picking" in an infected flock should be regarded as a hazard to an eradication program for such a flock.

Flock conditions may increase the spread of the disease by means of the egg. Inadequate nesting facilities, which may cause birds to crowd in the nest or lay their eggs on the dropping boards and floor, result in an increase in the number of broken eggs. Pullets reaching sexual maturity frequently lay soft-shelled and hard-shelled eggs on the floor and dropping boards. The production of thin-shelled eggs may follow

outbreaks of certain diseases. All these factors are of great significance in the spread of pullorum infection in an adult flock.

The excreta of infected birds must be considered a means by which infection may spread to noninfected birds in a flock and also from one farm to another. Kerr (1930) and Van Roekel et al. (1935) have reported the recovery of the organism from the feces of hens. Van Roekel et al. (1932, 1935) observed that fecal transmission of the disease among semimature and adult stock apparently occurs very infrequently. Transmission was observed only after force feeding repeated doses of feces from infected hens. The dissemination of the disease in an adult flock by means of feces or litter contaminated with S. pullorum may be influenced by the numbers of organisms eliminated and the persistency of such elimination together with suitable environmental conditions. Such factors constitute a major problem in the eradication of the disease in a short interval retesting or multiple testing program of an infected flock. Contamination of the exterior of the egg with feces and litter containing S. pullorum and the penetration of the organism through the shell may appear plausible (Cantor and McFarlane, 1948; Stokes et al., 1956).

Furthermore, cannibalism in an infected flock may further the spread of the disease. The abdominal viscera of infected birds in many instances are heavily contaminated with S. pullorum, and when such birds are eviscerated through cannibalistic habits in a flock, they may serve as a source of infection for other birds in the flock.

The important discovery of the organism in the egg established one phase in the cycle of infection. The most frequent spread of the disease occurs from the breeding female to its progeny by way of the egg. At the present time, this is the most common mode of transmission and will continue to be the case if infected birds are tolerated in a breeding flock. It is recognized that the greater the number of infected birds in a breeding flock, the greater will be the number of infected chicks. It has also been observed that one or two infected breeding birds may be responsible for serious infection in the progeny.

Transmission of the disease in incubators through chick excretions, egg shells, and chick down has been recognized as a very serious problem in the control of the disease for many years. Hinshaw et al. (1926) definitely revealed that artificially contaminated chick down could disseminate the disease in a forced-air-draft incubator. It was emphasized that in commercial hatching, precautions should be exercised against the spread of the infection through the incubator. Bunyea and Hall (1929) pointed out that pulmonary and cardiac lesions appear to represent a form of the disease acquired by inhalation and that gross intestinal lesions, such as thickening and necrosis of the large intestine, are indicative of infection by ingestion. The significance of incubator transmission of the disease will be discussed later in this chapter.

From the time the chick is removed from the incubator to the time it is placed in a brooder, it may contract the infection through being handled or through contact with infected chicks in the same chick box. Droppings of infected chicks serve as the source of the infection. Chick boxes and any other equipment of a like nature should not be used a second time unless properly cleaned and disinfected. Chick handlers, and especially chick sexers, should give consideration toward reducing the spread of the infection when chicks from different flocks are handled.

The infection, as a rule, is spread among chicks in cohabitation during the brooding stage, especially during the first few days of age. According to Weldin and Weaver (1930), the chief source of the organism at this stage is the droppings. Mallmann (1929), through the use of a brilliant green enrichment medium, was able to isolate the organism from the feces of infected chicks. He observed that when S. pullorum was in the organs of the chicks,

it was nearly always found in the intestinal contents. Litter, feed, and water soiled with infective droppings aid in the rapid spread of the infection in a chick flock. Botts et al. (1952) reported that *S. pullorum* disappeared more rapidly from old built-up litter than in new cob litter. Gwatkin and Mitchell (1944) found that pullorum disease could be produced in chicks which had access to feed contaminated by infected flies and to the flies themselves, some of which were probably eaten by the chicks. *S. pullorum* was recovered from the feet and wings of flies at least 6 hours after exposure. The gastrointestinal tract of the flies was found to harbor the infection for at least 5 days.

The mode of spread among young chicks is often facilitated through environmental factors such as extremes in temperature, insanitary conditions, lack of or inadequate feed, and other diseases appearing concurrently. It is frequently observed that chicks which originate from a hatchery not recognized as free from pullorum infection and which are subjected to unfavorable conditions in transit will manifest greater evidence of the disease than will chicks from the same source which are subjected to more favorable conditions. Proper brooder management plays a very important role in keeping the spread of infection down to a minimum.

Pullorum infection is likely to occur regardless of the portal of entry. Van Roekel et al. (1932) report that pullorum disease can be reproduced in chickens by dropping suspensions of the organism on the conjunctiva, into an incision in the skin of the plantar surface of the foot, into the cloaca, and by oral administration. However, the oral route did not yield to the establishment of infection as readily as others that were investigated. The presence of agglutinins was detected 6 days after cloacal inoculation, 7 days in the case of ocular route, exposure in the case of cloacal inoculation, 7 days with the ocular route, 10 days each for the oral route and skin incision instillation. In some cases, agglutinins were produced but later disappeared from the blood stream. Likewise, this might occur in a naturally infected flock. Some investigators (Bunyea and Hall, 1929; Doyle and Mathews, 1928; Hinshaw et al., 1926) reported that infection may result from the entrance of the organism into the respiratory and alimentary tracts. Apparently the portal of entry for posthatching infection is more often the digestive tract than the respiratory tract.

Hence, it appears that *S. pullorum* may be eliminated and excreted from the infected host in various ways and in turn may enter the body through various avenues when suitable environment exists. This is significant from the standpoint of control, eradication, and prevention of disease.

SYMPTOMS AND LESIONS

Adult fowl. Pullorum disease in a maturing or an adult flock does not manifest the characteristics of an acute infection as a rule. The spread of infection within a flock may occur continuously, but the flock owner may not be aware of it. In contracting the infection, the bird may exhibit little or no symptomatology. Infected individuals cannot, as a rule, be detected by their physical appearance (Fig. 8.4). Experimentally infected birds have exhibited limited and transient clinical manifestations. A general depression and listlessness, accompanied by a partial or complete inappetence, may be the first symptoms following the infection. A paleness of the comb and visible mucous membranes may be observed. Diarrhea may be noted. A febrile reaction accompanied by increased thirst has been observed. Occasionally adults may succumb to artificial infection depending upon the dosage and virulency of the organism.

Natural epornitics in a flock have been observed. Jones (1913a) reported a natural outbreak among adult fowl which was attributed to the feeding of eggs discarded from an incubator. Evidence of disease was observed 16 days after the feeding of the infective eggs, and during a period of 6 weeks a loss of 50 birds among a flock of 700 hens was sustained. The

FIG. 8.4 — Bird No. I, noninfected hen. Bird No. II, infected hen.

FIG. 8.6 — Pedunculated ovum obtained from an infertile egg containing an apparently normal yolk. S. pullorum was isolated from its contents. ×2.
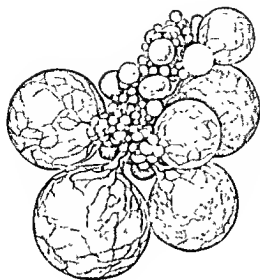
FIG 8.5 — Top, normal ovary. Below, infected ovary (S. pullorum).
(Storrs Agr. Exper Sta)

symptoms noted were paleness of the comb and mucous membranes; scaly, shrunken, and grayish appearance of the comb; listlessness; progressive depression; droopy wings, retraction of head and neck; inappetence; and usually diarrhea. The duration of the incubation period was from 16 days to 3 weeks. The course of the disease sometimes terminated fatally in 24 hours, usually continued 4 or 5 days, and occasionally even longer. A definite leukocytosis was observed.

Plastridge and Rettger (1930) have observed among adult flocks acute outbreaks of the disease caused by a highly pleomorphic type of *Salmonella pullorum*. Acute flock infections should not be mistaken for outbreaks of fowl typhoid, fowl cholera, avian listerellosis, and avian monocytosis.

Among birds dying from acute infection, Jones (1913a) observed marked emaciation; enlarged and distorted heart due to grayish-white nodules; enlarged, yellowish-green and granular liver coated with fibrinous exudate; friable spleen of normal size with focal necrosis; minute necrotic foci of the pancreas; enlarged kidneys with parenchymatous degeneration; injection of mesenteric vessels; and a fibrinous exudate coating the abdominal viscera.

The lesions found in the more common chronic carrier are the misshapen, discolored, cystic ova (Fig. 8.5), and frequently an acute or chronic pericarditis. The diseased ova usually contain oily and cheesy materials enclosed in a thickened isocapsule. The organism can be readily isolated from the ovarian cysts. These cysts may be closely attached to the ovary, but frequently they are pedunculated and may become detached from the ovarian mass. In such cases they become embedded in the adipose tissue of the abdominal cavity. In one case a pedunculated ovum was recovered as a foreign body from a normal-appearing egg (Fig. 8.6). Ovarian and oviduct dysfunction may lead to abnormal ovulation or oviduct impaction, which in turn may bring about extensive peritonitis

and adhesions of the abdominal viscera (Fig. 8.7). Advanced lesions of this type seldom, if ever, fail to yield *S. pullorum* on culture.

Lesions less extensive in nature may involve the heart. Quite frequently pericarditis is observed both among females and males. The changes that have occurred in the pericardium, epicardium, and pericardial fluid appear to be dependent on the age of the disease process. In some cases the pericardium exhibits only a slight translucency, and the pericardial fluid may be increased and possess a turbidity. In the more progressive stages the pericardial sac is thickened and opaque and the pericardial fluid is greatly increased in amount, containing considerable exudative material. This may be followed by permanent thickening of the pericardium and epicardium and partial obliteration of the pericardial cavity by adhesions (Fig. 8.8). The organism can usually be recovered from such a process.

In the male the infection is frequently found in the reproductive organs. Edwards and Hull (1929) reported the localization of the organism in the testicle and vas deferens. A thickening of the tunica albuginea and complete obliteration of the seminiferous tubules were observed. The testis revealed multiple small abscesses and areas of round cell infiltration. There was no evidence of spermatogenesis. The lumen of the vas deferens was enlarged and filled with a dense structureless homogeneous exudate. Other cases of infection in the male reproductive system have been reported (Fig. 8.9). Pericarditis is frequently observed among infected males, and less frequently small infective cysts containing amber-colored, cheesy material may be found embedded in the abdominal fat or attached to the gizzard or intestines.

Bushnell *et al.* (1926) reported the isolation of the organism from abscesses on the skin and the legs. Subcutaneous abscesses over the sternal region and cystic enlarged thyroids have yielded the organism. Gwatkin and Glover (1930) isolated

FIG. 8.7 — Impacted oviduct removed from an infected hen. Parts I and II are the funnel and albumen secreting portions, respectively. Part III is the shell gland portion. Adhesions of the serosa.

the organism from the nasal passages of 2 among 61 adult birds examined. Beach and Freeborn (1927) reported the isolation of *S. pullorum* from the middle ear with no evidence of infection in any other part of the body. Gwatkin (1946) recovered the organism from the thymus glands.

Chick. Manifestations of pullorum disease were first recognized among young chicks, and the malady may be considered as principally a chick disease. The symptoms exhibited by an infected brood of chicks are not specific for pullorum disease, although in many cases a tentative diagnosis based on clinical evidence has been substantiated by the isolation of the etiological agent.

In a typical outbreak of infection among chicks, the following symptoms may be observed: The onset of the disease varies with the degree, virulence, and source of the infection, and with the management given the chicks. If chicks are hatched from infective eggs, moribund and dead chicks may be observed in the incubator or within a short time after hatching. The chicks manifest a somnolence, weakness, and loss of appetite, and death may follow

FIG. 8.8 — (Left) Normal heart. (Right) Infected heart exhibiting pericarditis and epicarditis.

FIG. 8.9 — Testicles removed from a reacting adult male. Testis I—atrophic and very firm; S. pullorum reisolated. Testis II—normal in size and texture; S. pullorum not isolated.
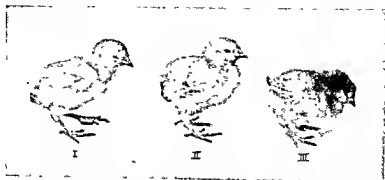
FIG. 8.10 — Nine-day-old, naturally infected pullorum disease chicks. S. pullorum was isolated from chicks II and III. Chick III died two days after it was photographed.

suddenly. In some instances, evidence of the disease is not observed until several days (5 to 10) after hatching. The disease gains momentum during the following 7 or 10 days. The peak of the infection usually occurs during the second or third week of chickhood. In such instances, the chicks exhibit a lassitude, an inclination to huddle together under the hover, loss of appetite, an accumulation of urinary and alimentary excretions in and around the vent, drooping of the wings, somnolence, and a distorted body appearance (Fig. 8.10). Frequently one may detect a shrill cry from a chick when voiding excreta. This is particularly true among those chicks that have an accumulation of whitish, chalklike excreta, stained greenish brown, in and around the vent.

Jungherr (1935) reports that affected chicks manifest a febrile reaction as indicated by the increased temperature of the legs. He also mentions that affected chicks may appear as having been "dipped in water" which he explained as probably brought on by a water-logged condition of the body muscles which permits the excess fluid to ooze through the skin. Hutyra et al. (1938) claim that a febrile condition is responsible for the increased renal activity and elimination of the whitish material adhering to the vent and adjacent parts.

Labored breathing may be observed even to the extent that chicks may be gasping for breath. This should not be confused with what is commonly designated "brooder pneumonia" or mold infection, and neither should it be mistaken for infectious bronchitis, Newcastle disease, or some other respiratory disturbance. The mortality may vary from no losses to approximately 100 per cent in serious outbreaks. The morbidity and mortality rates are dependent upon many factors. The greatest losses occur usually during the second week after hatching with a rapid decline during the third and fourth weeks. Survivors may be greatly retarded in their growth and appear as underdeveloped and poorly feathered birds (Fig. 8.11). However, some survivors may not reveal any great setback in growth, but grow to maturity even though harboring the infection. Chick flocks which have passed through a serious outbreak usually reveal a high percentage of carriers at maturity.

Evans et al. (1955) reported a blindness in chicks associated with salmonellosis. In one case S. pullorum was isolated from the anterior chamber of the eye and from the tibiotarsal joint. Fluctuating swellings of the tibiotarsal and the humeroradial and ulnar articulations, containing amber viscous exudate or a lemon-colored gelatinous material, have been ascribed to S. pullorum infection in chicks 2 weeks or older. Bacteriologic examination of the inflammatory process will yield pure cul-
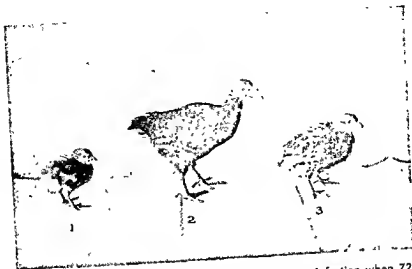
FIG. 8.11 — Six-week-old chicks exposed to pullorum infection when 72 hours old. Weights: No. 1, 115 gm.; No. 2, 488 gm.; No. 3, 193 gm.

FIG. 8.12 — (A) Pullorum-diseased heart exhibiting nodular abscesses in the myocardium (16-day-old chick). (B) Normal heart (17-day-old chick).
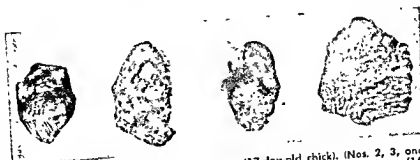
FIG. 8.13 — (No. 1 from left) Normal lung (17-day-old chick). (Nos. 2, 3, and 4) Pullorum-infected lungs exhibiting pneumonia and multiple abscesses (16-day-old chick).

tures of the organism. This arthritic involvement has been observed by several workers (Beaudette, 1936; Ferguson et al., 1961; Van Roekel and Smyser, 1962). Davis et al. (1961) referred to this clinical manifestation as a subcutaneous blisterlike lesion. One should not confuse this manifestation of pullorum disease with arthritic conditions produced by other infectious diseases.

Chicks hatched from an infected flock and raised on the same premises will usually reveal less mortality from the disease than chicks from the same flock shipped away.

In chicks that die suddenly in the early stages of brooding, the lesions are limited. The liver is enlarged and congested, and the normal yellow color may be streaked with hemorrhages. In the septicemic form, an active hyperemia may be found in other organs. The yolk sac and its contents reveal slight or no alteration. In the more protracted cases, an interference with yolk absorption may occur, and the yolk sac contents may be yellowish in color and of creamy and cheesy consistency. Necrotic foci or nodules may be present in the cardiac muscle (Fig. 8.12), liver, lungs (Fig. 8.13), ceca, large intestine, and the muscle of the gizzard. Pericarditis may be observed in certain instances. The liver may reveal punctiform hemorrhages and focal necrosis The spleen may be enlarged (Fig. 8.14) and the kidneys congested or anemic with ureters prominently distended with urates. The ceca may contain a cheesy core, sometimes tinted with blood, which should not be confused with a somewhat similar lesion encountered in coccidiosis The wall of the large intestine may be definitely thickened Frequently peritonitis is manifested. Doyle and Mathews (1928) report that the liver is the most constant seat of gross lesions and followed in order by the lungs, heart, gizzard, and ceca. Among chicks only a few days old, the lung lesions may consist only of a hemorrhagic pneumonia, whereas in older chicks small yellowish-gray nodules and areas of gray hepatization may be found.

FIG. 8.14—(A) Normal spleen (17-day-old chick). (B) Pullorum-infected spleen exhibiting marked enlargement (16-day-old chick).

The nodules in the myocardium may attain a size causing a marked distortion in the shape of the heart.

The histopathologic findings in young chicks affected with pullorum disease present features not markedly different from those in other infectious diseases. Doyle and Mathews (1928) state that in young chicks the livers show hyperemia, hemorrhages, focal degeneration, and necrosis (Fig. 8.15). They claim that the accumulation of endothelial leukocytes which replace the degenerated or necrotic liver cells is a characteristic cell reaction of the liver to S. pullorum infection. The histopathologic pulmonary lesions may consist of diffuse, acute congestion and hemorrhage in the early stages. Later, well-defined focal lesions appear which consist chiefly of a mononuclear infiltrating type of cell, serofibrinous exudate, and cellular debris. The larger lesions may involve several lobules, bronchioles, and bronchi terminating in necrosis.

The nodules in the myocardium and in the muscle of the gizzard represent largely an infiltration with mononuclear cells and degenerative changes of the muscle fibers.

The pulmonary and cardiac lesions may be considered of considerable diagnostic importance. However, for an accurate diagnosis of diseased specimens, the presence or absence of these findings should be confirmed by bacteriologic examination.

DIAGNOSIS

The identification of pullorum disease in maturing and adult stock should be made on the basis of serologic findings and

FIG. 8.15 — Liver revealing focal degeneration and necrosis, ×100.
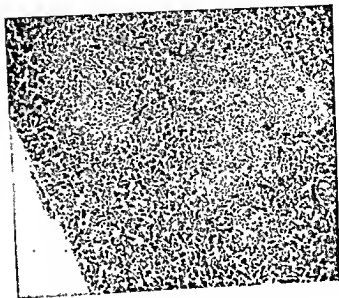
not on clinical and postmortem observations. Bacteriologic examination should be made in acute cases of the disease and in certain instances when the serologic findings may appear questionable. Other infectious diseases such as fowl typhoid, fowl cholera, paratyphoid infection, and avian monocytosis may at times be difficult to differentiate from pullorum infection without recourse to a bacteriologic examination. Disturbances of the female reproductive system are common in chickens, but pullorum disease can be incriminated for only a portion of them (Fig. 8.16). Serologic findings should not be considered final for all suspicious cases of pullorum disease. Fowl typhoid-infected birds and occasionally those harboring paratyphoid organisms will give a positive test when their sera are tested with pullorum antigen. Furthermore, it has been observed in routine pullorum testing that other bacterial organisms may cause birds to produce sera that will give nonspecific reactions with pullorum antigen (Garrard et al., 1947; Gwatkin, 1946). Nonspecific agglutination reactions for pullorum disease were observed with serum obtained from chickens infected with *S. enteritidis* var. *danysz*, an organism used in rat poison (de Blieck and Marthedal, 1952). Bacteriologic examination should be supplemented in such instances for an accurate diagnosis.

The diagnosis of the disease in young chicks should be based on bacteriologic examination even though well-pronounced lesions which may be considered quite characteristic of the infection are observed. Such a diagnostic procedure should definitely determine the presence or absence of fowl typhoid, paratyphoid, staphylococcosis, coccidiosis, infectious bronchitis, aspergillosis, and noninfectious diseases.

Standard Methods of Diagnosis of Pullorum Disease in Barnyard Fowl (Anon., 1933) adopted by the Conference of Official State and Federal Research Workers in Animal Diseases of America in 1931 and by the United States Livestock Sanitary Association in 1932 state that "the only criterion of infection with *Salmonella pullorum* shall be the isolation of this organism from the blood and body tissues of suspected chicks and its complete identification." Ordinary standard beef infusion agar is recommended for the isolation of the organism, and in case chicks have been in transit for some time, leading to partial decomposition of the specimens, brilliant green liver infusion medium as described by Mallmann (1929) is recommended. Various enrichment and selective media (tetrathionate broth, selenite broth, S-S,
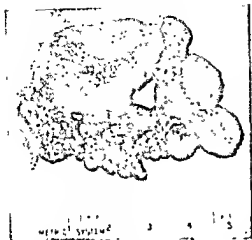
FIG. 8,16 — Cystic ovary infected with *Escherichia coli*.

and MacConkey agar) have been found useful in isolating the organism.

Cultures should be incubated from 21 to 48 hours at 37°C., and characteristic colonies should be identified by Gram-stained slide mounts and by their fermentative reaction in glucose, lactose, saccharose, and dulcitol. The antigenic specificity should be tested against known positive and negative pullorum sera. A Gram-negative medium rod, producing acid and gas, or acid in only glucose, and agglutinating with positive serum, is to be regarded as a typical criterion for *Salmonella pullorum*. The antigenic specificity may be tested with the rapid serum method, as recommended by Stafseth and Corbutt (1940), which may expedite completion of the examination and reporting the diagnosis to the consignee. Approximately 48 to 96 hours are required to obtain a diagnosis after the consignment arrives at the laboratory. Reliable and expeditious diagnostic service is essential because it may avoid serious losses and spread of the disease.

THERAPEUTICS

Until recently, efforts to reduce losses in outbreaks of pullorum disease through medicinal treatment have met with little or no success. Drugs and chemicals have been reported to have no beneficial influence when taken into the alimentary tract (Beach and Freeborn, 1927). Hypochlorite solutions were found to be of some value as a drinking water disinfectant. However, from a practical standpoint, considering the various other channels through which the organism may spread in a flock, it appears doubtful whether the expenditure for a drinking water disinfectant would be justified.

Introduction of sulfonamides in the control of the infection has revealed that sulfamerazine, sulfamethazine, and sulfaquinoxaline may be effective in reducing mortality from the disease. However, these drugs failed to prevent a retardation in growth associated with the disease. Likewise, the incidence of reactors among survivors was not reduced by administration of these drugs (Severens et al., 1945; Bottorff and Kiser, 1916; Gwatkin, 1946; Anderson et al., 1918; Cole, 1948; Dickinson and Stoddard, 1919; Grumbles et al., 1950; Cooper et al., 1951). The value of antibiotics in the control of pullorum disease is not known. Chang and Stafseth (1950a, 1950b) found that streptomycin was bactericidal and that this activity was enhanced by the use of sulfadiazine. Recently furazolidone, commonly known as nf-180, has been found to have some merit in preventing serious losses from the disease. The drug is administered in the feed at levels of .011 per cent and higher (Bierer, 1961; Gordon and Tucker, 1955; Henderson et al., 1958; Richey, 1962; Smith, 1951; Wilson, 1955, 1956; Smyser and Van Roekel, 1957, 1958).

The use of sanitary drinking fountains and feed hoppers, the frequent removal of contaminated litter, the maintenance of proper and uniform brooding temperatures, the avoidance of overcrowding, and the prompt removal of visibly sick and dead chicks are management measures which will reduce the spread of the infection and minimize losses.

Moore et al. (1934) have shown that increasing the brooder temperature from 5

to 10 degrees above that recommended for normal brooding operations will reduce the mortality. Bushnell *et al.* (1926) concluded that the feeding of sour milk is of little value in the control of pullorum infection, except that it may increase the vigor of the chicks due to its food value. Roberts *et al.* (1939a) reported that diet exerted an influence on the morbidity and mortality due to *S. pullorum*. A high mortality resulted from the feeding of laying mash, but when chick mash was substituted the high mortality disappeared.

Biotherapy (vaccines, bacterins, and serums) (van Heelsbergen, 1929) has been tried but has not given satisfactory results. According to Mallmann (1931a), phage therapy also has been ineffective in controlling the disease.

Incubator sanitation and disinfection are very essential in combating pullorum disease. At the start of hatching operations and repeated between hatches for the duration of the hatching season, incubators should be thoroughly cleaned and disinfected. Hatcherymen frequently overlook the fact that thorough cleaning ought always to precede disinfection. Liquid disinfectants and fumigants have their effectiveness greatly reduced if used in incubators containing chick down, eggshells, excreta, and other debris.

Formaldehyde, an extensively used fumigant, has been found very effective in incubator disinfection. Investigations (Bushnell *et al.*, 1929; Bushnell and Payne, 1931; Graham, 1941; Gwatkin, 1927; Insko *et al.*, 1941) have revealed that definite procedures must be followed to obtain the optimum results. It is generally recommended that 35 cc. of formalin be added to 17.5 gm. of potassium permanganate to fumigate 100 cubic feet of incubator space with wet-bulb and dry-bulb temperature readings of 86°–90° F. and 100° F., respectively. The exposure to the gas should be not less than 1 hour nor more than 3 hours.

Burton (1946) emphasizes that at least 150 cc. of formalin and 100 gm. of potassium permanganate be used to fumigate 100 cubic feet of inside incubator space. After 20 minutes' exposure to the gas, complete destruction of *S. pullorum* was observed. It is stressed that factors such as air leakage, improper humidity and temperature, circulation of gas within the incubator, and duration time of fumigation all play a very important role in effective fumigation of the incubator. Considerable leakage of gas was found in some commercial machines. Earlier Graham (1941) reported that maximum results of fumigation (using 35 cc. of formalin and 17.5 gm. of potassium permanganate for each 100 cubic feet) may be expected only if incubators are relatively clean and if the doors of forced-draft incubators are kept closed for a minimum of 3 hours following the release of the formaldehyde. The gas may then be liberated from the machine either by opening the doors for a few minutes or by neutralizing with strong ammonia water. The ammonia water may be sprinkled on the walls of the inside chamber. In forced-draft incubators, the diffusion of gases is very rapid and effective; however, first the proper temperature and humidity should be established. Insko *et al.* (1941) advise raising the wet-bulb reading to 92°–94° F. at the time of fumigation and maintaining the dry-bulb thermometer at normal operating temperature. Fumigation at high concentrations during the first 3 days of incubation is advised against, because the embryos at this period can tolerate less formaldehyde than at a later age. Losses of practical significance were not observed until four times normal concentration (35 cc. formalin per 100 cubic feet of space) of the fumigant was used.

When eggs are hatched in separate hatching compartments, they should be fumigated on the eighteenth to twentieth day of incubation. Fumigation may be repeated at short intervals during the hatching process but should not be delayed until after the chicks have dried. The relative humidity should be maintained at a high level, which will aid the chick in withstanding the gas. The incubator should

remain closed for 8 to 10 minutes. Immediately after each fumigation all chicks, whether wet or dry, should be removed from the incubator and kept in a comfortable environment. Ammonium hydroxide may be used to advantage in neutralizing the formaldehyde and in facilitating the handling of the chicks.

In addition to the formalin and potassium permanganate method, the use of cheesecloth saturated with formalin has been recommended. This latter method may be more economical in the use of formalin, but it requires a longer fumigation period. Commercial fumigants (Graham, 1941) are also available on the market, but the public should refrain from using such preparations until they are endorsed by qualified authorities.

Effective incubator fumigation by the formaldehyde method cannot be expected unless proper conditions prevail, and even then certain limitations exist as in the case of fumigating chicks in the process of hatching Complete destruction of S.

pullorum does not occur, but it is possible to reduce the chances of infection to some degree within the incubator. Incubator fumigation should be regarded as only one step or means that may be employed to advantage in a program designed for the control, eradication, and prevention of the disease.

CONTROL AND ERADICATION

Rettger *et al.* (1914) first reported that they had definitely established the complete cycle of infection and that chicks which were infected with the organism when small may develop into permanent carriers and be a constant source of danger to young and old stock. They found that the carrier condition might be established in approximately 25 per cent of an infected flock. To combat the disease successfully, these investigators stated that the infection cycle should be broken. Attempts were made to detect the carriers in flocks by the recognition of diseased ovaries in birds that were slaughtered for meat. This

FIG. 8.17 — Macroscopic tube agglutination test. (Left), Negative reaction. (Right), Positive reaction

method was found inadequate and impractical in eliminating all infected birds from the flock.

A later method, the bacteriologic examination of fresh eggs from an infected flock, was also found impractical, unreliable, and costly in the eradication of disease.

Jones (1913b) reported the use of the macroscopic tube agglutination test as a means of detecting carriers of the infection and recommended the lower serum dilutions (1:50, 1:100, and 1:200) for routine testing (Fig. 8.17). The test was considered to be valuable in detecting infected fowl, but its practicability was dependent upon the value of the breeding flock.

The important contribution by Jones must be regarded as the foundation work for the diagnostic procedures which are in use at the present. Gage et al. (1914), and Rettger et al. (1915) substantiated the results of Jones and later employed the test in statewide campaigns with the objective of detecting flocks free from the disease as well as eliminating the disease by the detection and removal of the reactors.

In 1914 Rettger et al. (1915) tested 107 flocks representing 14,617 birds which revealed 9.85 per cent reactors. Among 786 males tested only 2.9 per cent reacted. As to range of infection in terms of percentage, the tested flocks were classified as follows: 0 per cent, 28; 1–10 per cent, 34; 11–20 per cent, 18; 21–30 per cent, 10; 31–40 per cent, 10; 41–50 per cent, 4; 50+ per cent, 3. The White Leghorn flocks revealed a lower positive flock incidence than did the heavier breeds (57.1 per cent White Leghorns, 84.6 per cent Barred Rocks, and 88.4 per cent Rhode Island Reds).

The results obtained from the practical application of the macroscopic tube agglutination method revealed that a single test is not sufficient, as a rule, for the complete elimination of infected birds from a flock. However, improvement in hatchability and livability was observed among eggs and chicks, respectively, produced by

tested flocks. It was recommended that only nonreacting birds be used for breeding purposes.

The initial testing of flocks in Massachusetts revealed results comparable to those reported in Connecticut. It is of interest to note that pullorum disease-free flocks did exist, while other flocks might reveal a large percentage of reactors.

As the testing programs expanded and additional states adopted the routine testing of flocks, the test itself was gradually modified and improved as to its technique. Various investigators, organizations, and federal and state agencies have contributed to the serologic diagnostic methods employed at the present time. The National Poultry and Turkey Improvement Plans (1963) endorse three methods [(1) The Standard Tube Agglutination Test; (2) The Stained-Antigen, Rapid Whole-Blood Test; and (3) The Rapid Serum Test] for official testing. The choice of method is determined by many factors and objectives peculiar to a state or a region. In some states the macroscopic tube agglutination method is considered the only official method for testing of flocks. In other states the rapid serum method and the tube test are regarded as official. In many states all three methods may be employed, but the whole-blood test is the one most generally used.

The techniques and procedures for the three official methods are described in detail by the Animal Husbandry Research Division, Agricultural Research Service, United States Department of Agriculture, Washington, D.C. (The National Poultry and Turkey Improvement Plans 1963). A brief review of each method is as follows:

THE STANDARD TUBE AGGLUTINATION TEST

The blood samples shall be collected by a properly qualified and authorized person. Suitable blood tubes, shipping containers, and bleeding and leg-banding equipment should be furnished by the diagnostic laboratory or the authorized agency in

FIG. 8.18 — Collecting blood samples for laboratory test.

FIG. 8 19 — Incising the median vein of the wing (Vena cutanea ulnaris).

FIG. 8.20 — Collecting the blood into a numbered etched tube.

charge of the testing program (Fig. 8.18). Blood tubes should be thoroughly cleaned and heated in a hot-air sterilizing oven. Cork stoppers should be boiled or washed and dried in a hot-air drying oven. Shipping containers for the blood samples should be constructed to permit washing and disinfection.

All birds tested are to be officially leg banded. The blood tube is identified with the leg-band number which is inscribed on the etched portion of the tube or on a gummed label. A small amount of blood (½ to 2 cc.) is collected from the median vein of the wing (Vena cutanea ulnaris) by incising the latter with a sharp-pointed lancet or knife (Figs. 8.19 and 8.20). The tube is laid on its side, permitting the blood to clot in a long slant. After the blood has coagulated, the samples are packed in containers designed for shipment by mail, express service, or special messenger to the laboratory. In extremes of temperature, precautions should be taken against freezing or overheating because the blood samples should arrive at the laboratory in a fresh state and unhemolyzed condition for a satisfactory test. All hemolyzed or spoiled samples should

be rejected. The diagnostic laboratory should be equipped with proper and adequate refrigeration facilities where blood samples should be retained until the sera have been tested and results of the tests are known. Occasionally, a retest on the same serum may be necessary to determine the pullorum status of a bird.

The antigen for the tube test should be prepared from representative strains of S. pullorum which are known to contain the different antigenic components normally found in S. pullorum (Edwards and Bruner, 1916). Furthermore, the strains should possess high agglutinability with positive serum but should not agglutinate with negative or nonspecific sera. The following pullorum strains are widely used for the official tube test antigen with satisfactory results: strain 17 isolated in 1916; strain 19 isolated in 1911; strain 20 isolated in 1917. All strains are of the "standard" type and were originally isolated by Doctor Leo F. Rettger (Williams and MacDonald, 1955). Stock cultures of the antigen strains should be grown and maintained on nutrient agar medium composed of dry granular agar (Difco) 2.0 per cent, Bacto peptone (Difco) 1.0 per cent, beef extract

(Difco) 0.4 per cent, and water. The final hydrogen-ion concentration should range from 7.0 to 7.2. The cultures should be transferred not more than once a month. Seed cultures should be taken from the stock strains rather than from rapid serial transfers in order to avoid contaminants or possible variation in the characteristics of the organism. Large test tubes, Kolle flasks, or Blake bottles containing nutrient agar medium may be used for producing the antigen. After 48 to 72 hours' incubation, the growth is washed off with sufficient phenolized (0.5 per cent) saline (0.85 per cent) solution to produce a very concentrated suspension. This suspension is filtered through sterile absorbent cotton or glass wool into sterile glass-stoppered bottles. The washings for each of the three strains are combined in equal volume-density, and the stock antigen is stored at 8°–10° C.

An alternate medium, designated thiosulfate glycerin and frequently referred to as TG medium, may be used to prepare antigen. It is claimed that this medium provides an antigen of excellent specificity and greatly increases the yield of the antigen from a given volume of medium (Williams and MacDonald, 1955).

For routine testing, a dilute antigen is prepared from the stock antigen by diluting the latter with physiological saline solution containing 0.25 to 0.5 per cent phenol. The turbidity of the antigen corresponds to 0.75–1.00 on the McFarland nephelometer scale, and the hydrogen-ion concentration is adjusted to pH 8.2–8.5 by the addition of dilute sodium hydroxide. The dilute antigen is prepared each day in order to reduce dissolution and plasmolysis to a minimum at the specified hydrogen-ion concentration.

The amount of diluted antigen employed in individual tests may vary from 1 to 2 cc.; however, the amounts should be constant and placed in clean, clear test tubes. Commercial devices are recommended for this phase of the work. The sera are added to the test tubes containing the antigen with a serologic pipette or a serum-delivery device which is accurately calibrated to deliver definite amounts. The maximum dilution employed must not exceed 1:50 and, according to available data, the 1:25 dilution appears to be the most efficient. After the serum and antigen are well agitated, the mixture should be incubated for at least 20 hours at 37° C.

The results of the tests are interpreted as follows: *Negative test* represents a test in which the fluid remains uniformly turbid. *Positive test* represents a test in which the antigen reveals a distinct clumping, and clumps of cells have settled to the base of the tube with the supernatant fluid being clear. Gradation of clumping or agglutination may occur between negative and complete positive tests. These may be designated as slightly and strongly suspicious.

All suspicious and positive reacting tests should be reported to the agency responsible for the disposition of infected birds. Also, all broken, missing, and spoiled samples should be reported. In case the past status of the flock has been free of infection and only a few reactors are detected, the serologic diagnosis should be confirmed by bacteriologic examination of the reactors. An approved bacteriologic examination procedure for reacting birds has been prescribed for official testing (The National Poultry and Turkey Improvement Plans, 1963). Such a procedure will avoid a false diagnosis of fowl typhoid or paratyphoid infections. If only suspicious reactions are observed in a flock, then the strongest reacting birds should be submitted to the laboratory for retesting and a careful bacteriologic examination. In routine testing, flocks should not be condemned as infected on the basis of doubtful or atypical reactions because such reactions may be due to causes aside from *S. pullorum*. If no conclusive evidence of pullorum infection can be found, the flock should be regarded as negative. This statement is based on observations made in routine testing in the New England States (Van Roekel and Bullis, 1937). The lowering or removing of the official pullorum

status of a flock should be exercised only after conclusive evidence of infection has been established.

THE STAINED-ANTIGEN, RAPID WHOLE-BLOOD TEST

The stained-antigen, rapid whole-blood test was first developed by Schaffer et al. (1931), and Coburn and Stafseth (1931). At the present time, the antigen for this method is produced under federal license from the Secretary of Agriculture, in accordance with specific directions.

For a detailed description of the official procedure, reference should be made to the revised report on The National Poultry and Turkey Improvement Plans (1963). Briefly, the method may be described as follows: A wire loop, three-sixteenths of an inch in diameter made on the end of a 2½-inch length, noncorrosive wire (Brown and Sharpe gauge No. 24), is used to measure the blood. One end of the wire is inserted into a cork stopper which serves as a handle. A loopful of blood is taken from the punctured wing vein and contains approximately .02 cc. when the blood appears to bulge out. The loopful of blood is mixed with the stained antigen which has been placed on

a glass plate marked off in inch squares. The antigen is measured with a medicine dropper whose tip is constructed to deliver .05 cc. when held in a vertical position. An antigen-blood dilution of two to one or three to one has been reported to give the most satisfactory results. The loopful of blood is mixed with the antigen, and the mixture is spread out about an inch in diameter. The loop is washed in clean water and dried with cheesecloth or blotting paper. The glass plate is tilted up and down several times to aid in the mixing of the blood and antigen and apparently has some influence on the speed of the agglutination. Reactions may occur within a few seconds up to 2 minutes. Delayed reactions should be regarded as nonspecific. A positive reaction consists of the clumping of the violet-stained cells floating in clear fluid (Fig. 8.21). The rapidity of the reaction and the size of the clumps are influenced by the agglutinating power of the blood. Partial reactions should be regarded as suspicious and treated in the same manner as those observed in the other testing methods. Sometimes a very fine granulation appears which should be considered negative. Very infrequently agglutination of the red blood cells occurs and should not be confused with the
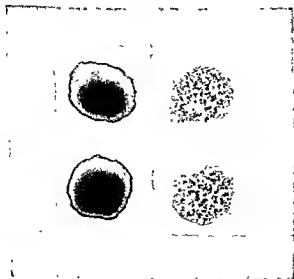
FIG. 8.21 — Stained-antigen, whole-blood test. (Left) Negative reaction. (Right) Positive reaction.

clumping of the stained bacterial cells. The fine marginal flocculation, which may be observed before drying of the mixture, is to be considered negative. A negative *reaction is one in which the mixture remains homogeneous for at least 2 minutes.* MacDonald (1917) developed a colloidal-sulfur medium (K) for the production of antigen. This medium produces an antigen which is superior to other stained antigens provided previously. Since July 1, 1953, all whole-blood crystal violet–stained antigen has been produced according to the K formula and is known as K antigen (Williams and MacDonald, 1955). Since July 1, 1957, all antigens have been of the polyvalent type which contains both "standard" and "variant" type strains (The National Poultry and Turkey Improvement Plans, 1963).

Only those reactions which appear within 1 minute after the mixing of the blood and antigen should be considered definitely positive, while reactions delayed for 2 minutes should be considered suspicious.

In order to approach uniformity of results, the testing plate should be well lighted at all times, and the temperature should remain at a constant level. A temperature of 75°–85° F. is considered satisfactory. The test plate should be free of dust and so constructed that it can be tilted with ease. The tested birds can be retained in either special holding equipment or crates and released as rapidly as the results of the test become known (Fig. 8.22). All birds in the tested flock should be officially leg banded. The accuracy of the results is greatly influenced by the competency of the testing agent and his thoroughness and care in conducting the test.

THE RAPID SERUM TEST

The rapid serum test for the detection of pullorum disease carriers was developed by Runnells *et al.* (1927). The blood samples may be collected in a manner similar to that described for the tube test. The antigen employed should consist of representative strains of *S. pullorum* which are of known antigenic composition and high agglutinability, but which are not

FIG. 8.22 – Conducting the stained-antigen, whole-blood test in the poultry house.

sensitive to negative and nonspecific sera. The strains are suspended in 12 per cent sodium chloride solution containing 0.5 per cent phenol. The turbidity is adjusted to 50 times greater than tube 0.75 of McFarland's nephelometer.

A box with a glass top ruled off in inch squares and improvised with lighting and heating facilities was used for testing. Two serum-antigen dilutions corresponding to the 1:50 and 1:100 dilutions for the tube test were employed. The amounts of serum used were 0.02 cc. and 0.01 cc. to which was added 0.02 cc. of antigen. The serum and antigen were mixed thoroughly with a toothpick. Positive reactions may occur quickly, but delayed reactions may require several minutes (Fig. 8.23). Gradations of reactions occur in this method as in other methods. Considerable experience is necessary for proper interpretation. This method should be used only in competent hands if the results are to be regarded as official. The results of the tests and the numbers of spoiled, broken, and missing samples should be reported directly to the flock owner or the agency in charge of the field work.

MISCELLANEOUS TESTS

Other tests, including the intradermal, precipitin, and complement-fixation methods, were found to be unreliable and impractical for the control and eradication

of the disease (Ward and Gallagher, 1917; Bushnell and Brandly, 1929; Edwards and Hull, 1929; Michael and Beach, 1929; and Rettger et al., 1930).

Williams (1951) has described a so-called spot test for the detection of infected chickens. This test may serve as a useful adjunct to the agglutination test, especially in flocks in which suspicious reactors are detected.

Limited observations have been reported concerning a flocculation test for pullorum disease by Roznowski and Foltz (1958).

In the control and eradication of the disease, the actual detection of infected birds through testing is only an integral part of a large program. A testing program which does not consider all the ramifications of pullorum disease is doomed to meet failure. Indiscriminate testing for the purpose of advertising or promoting the sale of stock should be prohibited. An adequate testing program should give consideration to the following eradication and preventive measures:

1. All birds over five months of age should be tested annually in order to determine the true status of a flock. When infection exists in the flock, partial flock testing is not as effective in eliminating the disease from the premises as is 100 per cent testing.

On commercial farms where large units of birds are maintained for egg production
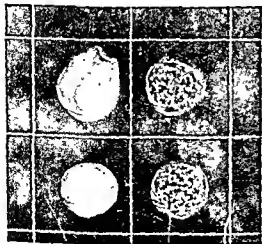
FIG. 8.23 — The rapid serum agglutination test. (Left) Negative reaction. (Right) Positive reaction.

in addition to units held for breeding purposes, the testing of only the breeding stock might suffice provided proper facilities exist for the segregation of the two groups and adequate precautionary measures are carried out against direct or indirect contact between the two groups. One should not consider a plant having both untested and tested birds as safe as a plant with only tested birds. A keen and careful buyer of stock will always carefully investigate this point and buy only from a flock whose status is without doubt.

Intermittent testing, that is, testing one year and not the next, or on alternate years, is a procedure which is not effective in establishing or maintaining a free flock. Those engaged in an official testing program should adopt the policy of minimizing such a practice.

2. Flocks revealing infection should be retested within two or four weeks until a negative report is obtained provided the value of the birds justified the expenditure. In the majority of cases, infection can be eliminated from a flock through short interval testing. Two or three retests in many instances are sufficient to detect all the infected birds in a flock (Table 8.1). Occasionally, infection may be very persistent, so that its elimination may not be accomplished by a testing program.

A retesting program for an infected flock or flocks should be complete to the extent that all infected birds have been detected and removed. Permitting one or a few infected birds to remain in a flock after a partial retesting program has been applied may lead to the propagation of the infection in the progeny of that flock which will necessitate a program of multiple testing from year to year to combat the disease. The objective should be to eliminate all of the infected birds from the breeding flock and reduce the cost of testing to one annual test in order to determine the status of the flock. In areas where the majority of flocks are free of the disease, the need for multiple retesting has been eliminated, which consequently represents a great economy to the poultry industry.

Infected flocks of inferior breeding or revealing heavy infection should not be considered for retesting. Replacements from known pullorum-clean sources will prove more effective and less expensive than intensive retesting for the establishment of a free flock. However, the pullorum disease-free stock selected for replacements should be protected against reinfection, which is not always fully appreciated. In some states the policy of replacement of infected flocks with stock from free sources has contributed more to the eradication of the disease and at less cost than could have been accomplished through retesting. In areas where an appreciable number of flocks still harbor the infection, it would behoove the official state agencies, commercial hatcherymen, and flock owners to carefully consider ways and means whereby similar progress can be made in their respective localities. Once the goal of pullorum freedom has been attained, a retrogression to pullorum-infected flocks will not be tolerated.

3. Every reactor, regardless of its value, should be removed from the premises and sold for slaughter immediately upon the completion of the test. When reacting birds are submitted to the laboratory for necropsy to confirm the results of the test, the recommended minimum procedure for bacteriologic examination should be followed (The National Poultry and Turkey Improvement Plans, 1963). Reactors should not be retained or sold for egg production because they would serve as sources for the spread of the disease.

4. The poultry houses, runs, and equipment should be thoroughly cleaned and disinfected immediately after removal of reactors. Disinfectants approved by federal or state agencies are recommended. Thorough cleaning of pens and other contaminated areas on the premises is most essential to attain eradication. Van Roekel et al. (1941) have observed that S. pul-

TABLE 8.1
RETESTING DATA OF TEN INFECTED FLOCKS

Flock Number		First Test	Second Test	Third Test	Fourth Test	Fifth Test	Results of Subsequent Season
1.	No. of birds tested	189	152	48	. . .	.	467
	Percentage reactors	1 59	0 00	0 00			0.00
2.	No. of birds tested	369	256	218			232
	Percentage reactors	0 54	0.39	0.00			0.00
3.	No. of birds tested	125	98	91			201
	Percentage reactors	20.00	4.08	0 00			0 00
4.	No. of birds tested	243	262	223	179		199
	Percentage reactors	11 11	1.15	0 00	0.00		0 00
5.	No. of birds tested	464	444	433	397		1,087
	Percentage reactors	2.37	0 45	0.00	0.00		0.00
6.	No. of birds tested	1,765	1,559	1,508	1,108	767	1,796
	Percentage reactors	3.17	0.13	0.00	0.00		0.00
7.	No. of birds tested	2,079	1,929	1,811	1,648	1,337	2,132
	Percentage reactors	3 17	1 09	0 00	0.00	0 00	0 00
8.	No. of birds tested	704	691	610	422		693
	Percentage reactors	8.24	8 83	0.16	0 00		0 00
9.	No. of birds tested	2,722	2,413	2,284	1,929		3,707
	Percentage reactors	1 80	0 54	0.48	0.00		0 00
10.	No. of birds tested	640	440	399	352	339	747
	Percentage reactors	27.34	4 32	1 00	0.00	0.00	0.00

lorum may remain viable on a dry cloth maintained at room temperature for at least 7 years and 8 months. Allen and Jacob (1930) found the organism to persist in a virulent condition for at least 14 months in samples of contaminated soil and deduced from this that the infection could exist on the premises from one season to the next. Kerr (1930) observed that S. pullorum remained viable in fecal emulsions for more than 3 months. It appears that soil in runs or yards inhabited by infected flocks should be considered unsafe for pullorum-free stock. Placing such stock on new ground is a highly effective means against contracting the disease from contaminated soil. Frequent plowing and liming of contaminated soil will aid in the destruction of the organism.

5. Offal from all birds dressed for market or home consumption, as well as dead birds that are unfit for consumption, should be burned. Feeding of garbage should be avoided.

6. Eggs should not be saved for hatching until after a flock has been tested and found to be free of the disease. As long as infected birds are detected in a flock, one may anticipate losses from the disease among the progeny.

7. Fresh and infertile eggs from unknown or infected sources should not be fed to chickens or exposed to animals such as crows, sparrows, rats, and skunks which may carry or spread the infection. Investigations (Van Roekel et al., 1932) definitely reveal that infection may be contracted from such sources.

8. Poultrymen should not custom batch for untested or infected flocks (including fowl other than chickens) in view of the fact that the infection can be transmitted

through the incubator. This likewise applies to commercial hatcheries. Only hatching eggs from officially recognized clean flocks should be selected by commercial hatcherymen. Ample pullorum-clean stock is available to all hatcherymen, and there should be no compromise to hatch known infected stock. The hazard of transmission is too great. If this is not recognized and observed, progress in pullorum disease eradication will be greatly delayed.

9. Vigilance in the purchase of stock in the form of adults, chicks, and eggs from clean sources will reduce the number of "breaks" among tested flocks, and also will lessen the incidence of pullorum disease among specimens submitted to the laboratory. Official state agencies or their published lists of pullorum-free flocks should be consulted as a guide in the purchase of stock. Purchases should not be made on advertisements or sales literature alone, because of the lack of information or misleading statements. In recent years the production of started pullets has become a general practice. Frequently birds raised on different premises and originating from different breeder flocks are placed together on one farm as breeders. In numerous instances one is unable to trace the history

of the birds, and if a pullorum "break" occurs it is impossible to trace the source of the infection. This involved and complicated rearing practice adds further difficulty to an eradication program.

10. Birds removed from the premises to egg-laying contests and exhibitions should be held in quarantine and determined free of the disease before they are readmitted to the flock. In several instances the source of pullorum infection was traceable to contests and shows. Birds returned to the premises should be tested immediately upon their arrival and retested after 30 days' quarantine. The safest procedure is not to return such birds to the breeding flock in order to avoid introduction of other diseases as well as pullorum disease. Exhibition and fancier breeding flocks should be tested and reveal no infection before they are permitted entry into a poultry show. Since these exhibition birds may enter several shows in one season, they could serve as potential spreaders if infected.

11. Fowl other than chickens should be considered as a possible source of infection. The testing of such fowl may aid in determining their pullorum status. The eggs from chickens and from fowl other than chickens should not be hatched

TABLE 8.2

PULLORUM DISEASE TESTING SUMMARY OF 14 STATES COVERING A 35-YEAR PERIOD*

Item	1927–28†	1939–40	1950–51	1962–63
Chickens on hand	....	73,875,000	93,179,000	76,174,000
Number of tests	735,851	5,032,290	13,798,239	11,352,111
Percentage of positive tests (based on birds)	5.05	1.56	0.21	0.0007
Number of negative flocks (100 per cent tested)	372	4,663	11,903	3,806
Number of breaks in negative flocks (100 per cent tested)	43	325	313	6
Number of pullorum-clean flocks	201	1,735	10,313	3,753
Number of birds in pullorum-clean flocks	112,605	1,565,957	9,952,416	10,974,278

* Includes Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia.
† Does not include North Carolina, Vermont, and Virginia.

TABLE 8.3

PULLORUM DISEASE TESTING SUMMARY OF UNITED STATES COVERING A 26-YEAR PERIOD[a]

Item	1936-37	1949-50	1962-63
Number of flocks	9,191	111,422	21,272
Number of birds	4,329,364	37,237,674	35,236,200
Percentage of positive tests	3.66	0.72	0.005
Birds in pullorum-clean flocks	257,577	13,302,642	33,517,824

[a] The National Poultry Improvement Plan, 1963.

simultaneously in the same incubator. This precaution will avoid the spread of paratyphoid infections as well as pullorum disease among the different species of fowl.

12. Used feed bags and other equipment that may have been exposed to or contaminated with infective material should not be used unless properly cleaned and disinfected. Dunlap (1931) reported transmission of the disease to chicks which were fed mash from an artificially contaminated bag. The chance of processed feeds introducing pullorum infection cannot be ignored and further investigation is needed concerning this phase of the problem.

13. Poultry vaccines should be prepared from embryonating chicken eggs that are selected from pullorum-clean flocks. Fowl pox vaccine prepared from infected eggs has been responsible for extensive pullorum infection in vaccinated breeding flocks. As few as ten organisms per cubic centimeter of vaccine were able to infect chickens (Anon., 1951).

Eradication appears possible. Phenomenal progress in pullorum disease control and eradication has been made in the past decade (Van Roekel, 1964). In all sections of this country certain states have reduced the incidence of pullorum infection to a very low level (Tables 8.2 and 8.3). Some states, especially in the northeast, have reported no reactors among the tested flocks (Table 8.4). Also, the incidence of infection among specimens submitted to the diagnostic laboratories has declined to a very low figure. A number of states have not identified pullorum infection in either tested flocks or among specimens

submitted for examination to the diagnostic laboratory (Tables 8.5 and 8.6). The records reveal that pullorum disease is gradually being eliminated from poultry

TABLE 8.4

PULLORUM TESTING DATA SUBMITTED BY STATES AND CANADIAN PROVINCES IN THE NORTHEASTERN CONFERENCE ON AVIAN DISEASES

State	Year	Birds Tested	Percentage Positive
Connecticut	1925	20,743	2.40
	1963	470,191	0.00
Delaware	1925	4,300	5.70
	1963	522,360	0.00
Maine	1921	2,730	22.30
	1963	1,558,928	0.00
Maryland	1927	3,725	21.00
	1963	337,195	0.00
Massachusetts	1921	24,718	12.50
	1963	589,005	0.00
New Hampshire	1926	35,237	2.50
	1963	442,676	0.00
New Jersey	1926	52,611	7.86
	1963	395,574	0.00
New York	1926	59,576	6.20
	1963	478,827	0.00
North Carolina	1932	64,702	4.02
	1963	3,788,963	0.0006
Nova Scotia	1929	2,041	7.00
	1963	68,145	0.00
Ontario	1928	15,000	8.00
	1963	1,253,128	0.00
Pennsylvania	1924	2,077	15.00
	1963	1,344,923	0.0008
Rhode Island	1925	8,175	6.97
	1963	54,642	7.40
Vermont	1928	8,555	7.40
	1963	101,216	0.00
Virginia	1925	13,000	20.00
	1963	1,031,514	0.006
West Virginia	1928	2,005	6.00
	1963	342,914	0.00

TABLE 8.5

Incidence of Pullorum Infection in 14 North-
eastern States as Detected Among Tested Flocks
and Consignments Submitted for Diagnosis

State	1958 F*	1958 D†	1960 F*	1960 D†	1962 F*	1962 D†
Conn.	3	8	3	1	0	0
Del...	0	2	16	5	0	0
Maine.	0	3	0	0	0	0
Md..	2	5	1	19	0	2
Mass.	3	3	5	5	0	1
N.H.	0	3	0	0	0	1
N.J.	16	4	3	15	0	7
N.Y	3	11	2	5	0	9
N.C	5	34	3	21	16	13
Pa.	12	21	4	21	1	13
R.I.	0	0	1	1	0	0
Vt.	1	0	2	2	0	2
Va.	10	21	18	12	1	2
W.Va.	0	0	1	2	0	0
Total	65	122	55	109	18	50

* Infected tested flocks, 1962–63 season.

† Number of positive diagnoses among consignments
submitted for diagnosis.

flocks and that the disease is amenable to
complete eradication.

The poultry industry and those agencies
which have been concerned with control
and eradication are to be commended for
the progress that has been made in re-
ducing the level of pullorum infection in
this country. This has been accomplished

on a limited voluntary basis. However,
since pullorum disease is amenable to erad-
ication it will require certain regulatory
measures implemented by state and federal
disease regulatory agencies to bring about
complete eradication on state and national
levels. Some states have in recent years re-
vised their disease laws in order to further
the control and eradication of pullorum
infection. Groups of states with the co-
operation of the federal government are
considering area plans for the eradication
of the disease.

The poultry industry can benefit from
the eradication programs applied to other
types of livestock. State and federal disease
regulatory agencies are actively engaged in
animal health programs and can give valu-
able assistance to the control and eradi-
cation program for pullorum disease. In
fact, a number of states have recognized the
wisdom of including poultry diseases in
their over-all animal disease control pro-
gram and have taken progressive steps to-
ward improving poultry health.

In view of the outstanding progress that
has been made in eliminating pullorum
disease from the nation's poultry flocks and
the increased interest manifested in total
eradication, it is hoped that all states will
strive toward the same goal. This will re-
quire the fullest cooperation from the in-
dustry as well as all other agencies that can
contribute to this goal.

TABLE 8.6

Incidence of Pullorum Disease Diagnoses Among Consignments Submitted to the
Diagnostic Services in 7 States

State	C,D*	1950	1954	1958	1962
California	C	3,282	3,670	10,390	14,584
	D	158	51	26	20
Delaware	C	3,241	3,855	1,053	4,373
	D	50	64	2	0
Indiana.	C	1,917	1,383	1,094	669
	D	215	111	58	18
Maryland	C	2,447	1,997	1,918	
	D	111	15		2
Minnesota	C	470†	200†	76†	1,335
	D	169	39	12	0
New Hampshire	C	1,701	2,549	1,893	1,041
	D	30	17	3	0
North Carolina	C	1,278	1,595	5,106	9,431
	D	119	117	37	29

* C = Consignments examined, D = Diagnoses of pullorum disease.

† Cases involving chicks 0–6 weeks of age.

REFERENCES

Allen, P. W., and Jacob, M.: 1950. Sodium acid sulphate as a disinfectant against *Salmonella pullorum* in poultry-yard soils. Tenn. Agr. Exper. Sta. Bul. 143.

Anderson, G. W., Cooper, J. B., Jones, J. C., and Morgan, C. L.: 1948. Sulfonamides in the control of pullorum disease. Poultry Sci. 27:172.

Anonymous: 1950. Eastern States Conference of Laboratory Workers in Pullorum Disease Control. Jour. Am. Vet. Med. Assn. 77.259.

Anonymous: 1953. Report of the conference of official research workers in animal diseases of North America on standard methods of pullorum disease in barnyard fowl. Jour. Am. Vet. Med. Assn. 82:487.

Anonymous: 1954. Report of the Committee on Transmissible Diseases of Poultry. Proc. 58th Ann. Meet. U.S. Livestock Sanit. Assn., p. 350.

Asmundson, V. S., and Biely, J.: 1930. Effect of pullorum disease on distribution of first year egg production. Scienc. Agr. 10:497.

Barboni, E.: 1937. Ricerche sul primo focolaio di pullorosi nei tacchini riscontrato in Italia. Clin. Vet. 60.507.

Beach, J. R., and Freeborn, S. B.: 1927. Diseases and parasites of poultry in California. Calif. Agr. Ext. Serv. Cir. 8, 5th ed., p. 110.

Beaudette, F. R.: 1936. Arthritis in a chick caused by *Salmonella pullorum*. Jour. Am. Vet. Med. Assn. 89.69.

——. 1938. Localized pullorum infection in the ovary of a duck. Jour. Am. Vet. Med. Assn. 92:100.

——, Bushnell, L. D., and Payne, L. F.: 1925a. Study of an organism resembling *Bacterium pullorum* from unaborted yolk of chicks "dead in shell." Jour. Infect. Dis. 32:124.

——, Bushnell, L. D., and Payne, L. F.: 1925b. Relation of *Bacterium pullorum* to hatchability of eggs. Jour. Infect. Dis. 33.331.

Belding, R. C.: 1955. The incidence of *Salmonella pullorum* in wild pheasants in southern Michigan. Poultry Sci. 34:1441.

Benedict, R. G., McCoy, E., and Wisnicky, W.: 1941. Salmonella types in silver foxes. Jour. Infect. Dis. 69:167.

Bierer, B. W.: 1961. A nitrofuran coccidiostat aids in control of pullorum disease under stress conditions. Jour. Am. Vet. Med. Assn. 139.238.

Bivins, J. A.: 1948. A survey of the incidence of serological variants of *Salmonella pullorum* in Michigan. Poultry Sci. 27:029.

Bottorff, C. A., and Kiser, J. S.: 1946. The use of sulfonamides in the control of pullorum disease. Poultry Sci. 25:357.

Botts, C. W., Ferguson, L. C., Birkeland, J. M., and Winter, A. R.: 1952. The influence of litter on the control of *Salmonella* infections in chicks. Am. Jour. Vet. Res. 13:562.

Bruner, D. W., and Moran, A. B.: 1949. Salmonella infections of domestic animals. Cornell Vet. 39:53.

Brunett, E. L.: 1930. Pullorum disease in the mature turkey. Poultry Sci. 9:356.

Bunyea, H.: 1939. An outbreak of pullorum disease in young guinea fowl. Jour. Am. Vet. Med. Assn. 94:233.

——, and Hall, W. J.: 1929. Some observations on the pathology of bacillary white diarrhea in baby chicks. Jour. Am. Vet. Med. Assn. 75:581.

Burton, W. H. J.: 1929. Control of pullorum disease transmission in hatcheries by formaldehyde fumigation. Proc. 16th Ann. Conf. Lab. Workers in Pullorum Disease Control, p. 1.

Bushnell, L. D., and Brandly, C. A.: 1929. Some experiments on the control of bacillary white diarrhea. Jour. Am. Vet. Med. Assn. 74:444.

——, Hinshaw, W. R., and Payne, L. F.: 1926. Bacillary white diarrhea in fowl. Kans. Agr. Exper. Sta., Tech. Bul. 21.

——, and Payne, L. F.: 1931. Dissemination of pullorum disease in the incubator. Kans. Agr. Exper. Sta., Tech. Bul. 29.

——, Payne, L. F., and Coon, C. J.: 1929. Fumigation of forced-draft incubators. Jour. Am. Vet. Med. Assn. 75 611.

——, and Porter, J. J.: 1945. A study of methods for the isolation of *Salmonella pullorum*. Poultry Sci. 24:212.

Cantor, A., and McFarlane, V. H.: 1948. Salmonella organisms on and in chicken eggs. Poultry Sci. 27:350.

Chang, Kuan-How, and Stafseth, H. J.: 1950a. Influence of various factors on the bacteriostatic and bactericidal action of streptomycin on *Salmonella pullorum*. Poultry Sci. 29.150

——, and Stafseth, H. J.: 1950b. Influence of serum of normal and pullorum-infected birds on the bacteriostatic and bactericidal action of sulfadiazine on *Salmonella pullorum*. Poultry Sci. 29:139.

Chute, H. L.: 1962. Personal communication

Coburn, D. R., and Stafseth, H. J.: 1931. A field test for pullorum disease. Jour. Am. Vet. Med. Assn. 79 241.

Cole, R. K.: 1948. Sulfonamides versus *Salmonella pullorum* in adult chickens. Poultry Sci. 27 427

Cooper, J. B., Morgan, C. L., Anderson, G. W., and Jones, J. C.: 1951. Effect of sulfamerazine on pullorum reactors. Poultry Sci. 30:249.

Dalling, T., Mason, J. H., and Gordon, W. S.: 1928. Bacillary white diarrhea (B.W.D.): B. pullorum isolated from sparrows. Vet. Record 8:323.

——, Mason, J. H., and Gordon, W. S.: 1929. Bacillary white diarrhea (B.W.D.): B. pullorum isolated from a turkey poult in England. Vet. Record 9:902.

Davis, D. E., Sims, H. M., and Buchanan, W.: 1961. A blister-like lesion associated with pullorum disease in broiler chicks. Southeastern Vet. 12:53.

Dearstyne, R. S., Knipp, B. F., and Wilfong, H. S.: 1929. Study of bacillary white diarrhea (pullorum disease). Agr. Exper. Sta. of N.C. State Coll. of Agr. and Engr., and N.C. Dept. of Agr. Tech. Bul. 36:53.

de Blieck, L., and Marthedal, H. E.: 1952. Investigations concerning the feeding of chickens with rutin in relation to the B.W.D. test. Vet. Record 64:815.

DeVolt, H. M., Quigley, G. D., and Byerly, T. C.: 1911. Studies of resistance to pullorum disease in chickens. Poultry Sci. 20:535.

Dickinson, E. M., and Stoddard, E. D.: 1919. Sulfamerazine against Salmonella pullorum in adult chickens. Poultry Sci. 28:513.

Doyle, L. P., and Mathews, F. P.: 1928. The pathology of bacillary white diarrhea in chicks. Purdue Univ. Agr. Exper. Sta., Bul. 323.

Doyle, T. M.: 1925. Bacillary white diarrhea of chicks. Jour. Comp. Path. and Therap. 38:266.

Dunlap, G. L.: 1951. Ann. Rep. Mass. Agr. Exper. Sta., Bul. 271:280.

E. A. H.: 1905. White diarrhea in brooder chicks. Farm Poultry 16, No. 9 256.

Edwards, P. R.: 1928. The fermentation of maltose by Bacterium pullorum. Jour. Bact. 15.235.

——: 1915. An outbreak of Salmonella pullorum infection in canaries. Jour. Am. Vet. Mrd. Assn. 107:245.

——, and Bruner, D. W.: 1913. The occurrence and distribution of Salmonella types in the United States. Jour. Infect. Dis. 72.58.

——, and Bruner, D. W.: 1916. Form variation in Salmonella pullorum and its relation to X strains. Cornell Vet. 30:316.

——, and Hull, F. E.: 1929. Bacillary white diarrhea and related diseases of chickens. Ky. Agr. Exper. Sta., Bul 296.237.

Emmel, M. W.: 1936. Pullorum disease in captive quail. Jour. Am. Vet. Med. Assn. 89.716.

Evans, W. M., Bruner, D. W., and Peckham, M. C.: 1955. Blindness in chicks associated with salmonellosis Cornell Vet. 45.259.

Felsenfeld, O., and Young, V. M.: 1944. The occurrence of members of the genus Salmonella in inhabitants of state hospitals of the greater Chicago area. Jour. Lab. and Clin. Med. 29.375.

Ferguson, A. E., Connell, M. C., and Truscott, R. B.: 1961. Isolation of Salmonella pullorum from the joints of broiler chickens. Canad. Vet. Jour. 2:113.

Gage, G. E., Paige, B. H., and Hyland, H. W.: 1914. On the diagnosis of infection with Bacterium pullorum in the domestic fowl. Mass. Agr. Exper. Sta., Bul. 148.

Garrard, E. H., Burton, W. H., and Carpenter, J. A.: 1917. Non-pullorum reactions. Proc. 19th Ann. Conf. Lab. Workers in Pullorum Disease Control, p. 1.

Gifford, E. G.: 1905. White diarrhea in incubator chicks. Farm Poultry 16, No. 10 259.

Gilfillan, R. F., Holtman, D. F., and Ross, R. T.: 1955. A synthetic medium for propagation and maintenance of virulent strains of Salmonella pullorum. Poultry Sci. 34 1285.

——, Holtman, D F., and Ross, R. T.: 1966a. Modification of pullorum disease in the chick by certain tricarboxylic acid cycle inhibitors and intermediates. Jour. Bact. 72.620.

——, Holtman, D. F., and Ross, R. T.: 1966b. Influence of Salmonella pullorum infection of various liver tricarboxylic acid enzymes and citrate levels in the chick. Jour. Bact. 72.624.

Gordon, R. F., and Tucker, J. F.: 1955. The treatment of chronic carriers of Salmonella pullorum with furazolidone. Vet. Record 67:116.

Graham, R. 1941 Hatchery sanitation and incubator hygiene. Proc. Conf. Natl. Poultry Improv. Plan, Bur. of Anim. Ind., U.S.D.A., 106

Graham, W. R.: 1904 White diarrhea in brooder chicks. Farm Poultry 15, No. 11:252.

Grumbles, L. C., Levy, Helen E., and Oglesby, W. T.: 1950. Sulfanamides in the control of pullorum disease in young chicks. Poultry Sci. 29 296.

Gwatkin, R. 1927 Some experiments on the disinfection of eggs and incubators. Ont. Vet. Coll. Rep (1926), p. 58

——. 1946. Resumé of studies relating to Younie strains of Salmonella pullorum. Proc. 18th Ann Conf. Lab. Workers in Pullorum Disease Control, p. 1.

——, and Glover, J. J.: 1930. Isolation of S pullorum from the nasal passages of two fowl. Ont. Vet Coll Rep (1929), p. 61.

——, and Mitchell, C. A:1944. Transmission of Salmonella pullorum by flies. Canad. Jour. Pub Health 35 281

Hanks, J. H., and Rettger, L. F.: 1932. Bacterial endotoxin. Search for a specific intracellular toxin in S. pullorum Jour Immunol. 22:283.

Harris, M. E., and Williams, J. E.: 1957. The hemagglutinating properties of Salmonella typhimurium Am Jour Vet. Res. 18.432.

Heemstra, L. C: 1948 The importance of the variant problem in pullorum disease control. Proc. 52nd Ann. Meet. U.S Livestock Sanit. Assn., p. 274.

Henderson, W., Walkey, F. L., and Morehouse, G. L.: 1958. Furazolidone treatment of experimental pullorum disease in adult chickens. Am. Jour. Vet. Res. 19:196.

Hendrickson, J. M.: 1927. The differentiation of *Bacterium pullorum* (Rettger) and *Bacterium sanguinarium* Moore). Jour. Am. Vet. Med. Assn. 70.629.

———, and Hilbert, K. F.: 1930. Report of the Poultry Disease Laboratory at Farmingdale, Long Island. Ann. Rep. N.Y. State Vet. Coll. (1928–29), p. 49.

———, and Hilbert, K. F.: 1931. Report of the Poultry Disease Laboratory at Farmingdale, Long Island. Ann. Rep. N.Y. State Vet. Coll. (1929–30), p. 51.

Hewitt, E. A.: 1928. Bacillary white diarrhea in baby turkeys. Cornell Vet. 18 272.

Hinshaw, W. R.: 1937. Diseases of turkeys. Univ. of Calif. Agr. Exper. Sta., Bul. 613.

———: 1939. Pullorum disease in turkeys. Proc. Conf. Nat. Poultry Improv. Plan, Bur. Anim. Ind., Anim. Hush. Div., U.S.D.A., p. 98.

———: 1941. Cysteine and related compounds for differentiating members of the genus Salmonella. Hilgardia 13, No. 11:583.

———, and Holman, H. A.: 1937. Pullorum disease in ducklings. Poultry Sci. 16:189.

———, Upp, C. W., and Moore, J. M.: 1926. *Studies in transmission of bacillary white diarrhea in incubators*. Jour. Am. Vet. Med. Assn. 68.631.

Hudson, C. B., and Beaudette, F. R.: 1929. The isolation of *Bacterium pullorum* from a European bullfinch (*Pyrrhula europa*). Jour. Am Vet. Med. Assn. 74.929.

Hutt, F. B., and Scholes, J. C.: 1941. Genetics of the fowl. XIII. Breed differences in susceptibility to *Salmonella pullorum*. Poultry Sci. 20:342.

Hutyra, F., Marek, J., and Manninger, R.: 1938. *Special Pathology and Therapeutics of the Diseases of Domestic Animals*. Vol. I, 4th English edition. Alexander Eger, Chicago, P. 212.

Insko, W. M., Steele, D. G., and Hinton, C. M.: 1941. Effect of formaldehyde fumigation on mortality of chick embryos. Kentucky Agr. Exper. Sta., Bul. 416.

Jones, A. W., and Holtman, D. F.: 1953. Synthesis of glutamic acid and alanine by *Salmonella pullorum*. Jour. Bact. 66.147.

Jones, F. S.: 1911. Fatal septicemia or bacillary white diarrhea in young chickens. Ann. Rep. N.Y. State Vet. Coll. (1909–10), p. 111

———: 1913a. An outbreak of an acute disease in adult fowls due to *Bacterium pullorum*. Jour. Med. Res. 27:471.

———: 1913b. The value of the macroscopic agglutination test in detecting fowls that are harboring *Bacterium pullorum*. Jour. Med. Res. 27:481.

Jungherr, E.: 1935. *Diseases of brooder chicks*. Storrs Agr. Exper. Sta., Bul. 202:56.

Kaupp, B. F.: 1917. *Poultry Diseases*. 2nd ed. Am. Vet. Pub Co., Chicago, P. 119.

Kerr, W. R.: 1930. Selective media for the cultivation of *Bacillus pullorum* and *Bacillus sanguinarium*. Jour. Comp. Path. and Therap. 43.77.

Lerche, M.: 1929. Ueber das Vorkommen der bakteriellen (weissen) Kückenruhr bei jungen Enten. Tierärztl. Rundschau. 35:160.

Luzio, A. J., Bushnell, L. D., and Erwin, L. E.: 1953. The antigenic variation of *Salmonella pullorum*. Poultry Sci. 32:7.

MacDonald, A. D.: 1947. K antigen for the detection of pullorum disease in poultry. Proc. 19th Ann. Conf. Lab. Workers in Pullorum Disease Control, p. 1.

Mallmann, W. L.: 1929. *Salmonella pullorum in the intestinal contents of baby chicks*. Jour. Infect. Dis. 44 16

———: 1931a. Studies on bacteriophage in relation to Salmonella and pullorum disease. Mich Agr. Exper. Sta., Tech. Bul. 109.

———: 1931b. Use of organic acids for the differentiation of *Salmonella pullorum* and *Salmonella gallinarum*. Proc. Soc. Exper. Biol. and Med. 28:501.

———: 1932. The dissociation of *Salmonella pullorum* and related species. Mich. Agr. Exper. Sta., Tech. Bul. 122.

———, and Snyder, D.: 1929. Differential medium for *Salmonella pullorum*, *Salmonella gallinarum*, *Pasteurella avecida*, and *Escherichia coli*. Jour. Infect. Dis. 44:13.

Marthedal, H. E.: 1952. Studier over pullorum-disease. Serologiske og kulturelle forhold samt antigen undersögelser. Nordisk Veterinärmed. 4 201.

Mathews, F. P.: 1927. Factors influencing the control of bacillary white diarrhea. Jour. Am. Vet Med. Assn. 71 583.

McCullough, N. B., and Eisele, C. W.: 1951. Experimental human salmonellosis. IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg Jour. Infect. Dis. 89:259

Michael, S. T., and Beach, J. R.: 1929. An experimental study of tests for the detection of carriers of *Bacterium pullorum*. Hilgardia 4, No 8 185.

Mizener, H 1951. Bacillary white diarrhea. Proc. Fourth World's Poultry Cong p. 428.

Mitchell, R B., Garlock, F C., and Broh Kahn, R. H.: 1946. An outbreak of gastro enteritis pre sumably caused by *Salmonella pullorum*. Jour Infect. Dis. 79.37.

Moore, J. M., Mallmann, W. L., and Arnold, L. R.: 1954. *Studies on pullorum disease*. I. The influence of different temperatures in brooding. Jour. Am. Vet Med Assn 84 526

Moran, A B.: 1961 Occurrence and distribution of Salmonella in animals in the United States. Proc 65th Ann Meet U S Livestock Sanit Assn., p 411

Mulsow, F. W.: 1919. The differentiation and distribution of the paratyphoid enteritidis group. VI. Avian paratyphoid bacilli: a comparative study of *Bacterium pullorum* and *Bacterium sanguinarium*. Jour. Infect. Dis. 25:135.

National Poultry Improvement Plan: 1941. U.S.D.A. Misc. Pub. 300-28.

———: 1957. U.S.D.A. ARS 44-5.

National Poultry and Turkey Improvement Plans: 1963. U.S.D.A., ARS, Misc. Pub. 739:39.

Olney, J. F.: 1928. *Salmonella pullorum* infection in rabbits. Jour. Am. Vet. Med. Assn. 73-631.

Pacheco, G., and Rodrigues, C.: 1936. O grupo pullorum-gallinarum em provas bacteriologicas comparativas. Inst. Osvaldo Cruz 31:591.

Plastridge, W. N., and Rettger, L. F.: 1930. An epidemic disease of domestic fowl caused by a hitherto undescribed organism of the *Salmonella pullorum* type. Jour. Infect. Dis. 47:334.

———, and Rettger, L. F.: 1932. Variants of *Salmonella pullorum*. Jour. Infect. Dis. 50:146.

Reis, J., and Nobrega, P.: 1936. Tratado de doenças das aves. Edição do Instituto Biologico São Paulo, Brazil, p. 105.

Rettger, L. F.: 1900. Septicaemia among young chickens. N.Y. Med. Jour. 71:803.

———: 1901. Septicemia in young chickens. N.Y. Med. Jour. 73:267.

———: 1909. Further studies on fatal septicemia in young chickens, or "white diarrhea." Jour. Med. Res. 21:115.

———: 1916. Occurrence and significance of *Bacterium pullorum* in eggs. Jour. Am. Assn. Instr. and Invest. Poultry Husb. 2 62.

———, Hull, T. G., and Sturges, W. S.: 1916. Feeding experiments with *Bacterium pullorum*. The toxicity of infected eggs. Jour. Exper. Med. 23:475.

———, Kirkpatrick, W. F., and Jones, R. E.: 1914. Bacillary white diarrhea of young chicks. (Fourth report) Storrs Agr. Exper. Sta., Bul. 77:259.

———, Kirkpatrick, W. F., and Jones, R. E.: 1915. Bacillary white diarrhea of young chicks; its eradication by the elimination of infected breeding stock. (Fifth report) Storrs Agr. Exper. Sta., Bul. 83:149.

———, McAlpine, J. G., and Warner, D. E.: 1930. A comparative study of the routine macroscopic agglutination and the intracutaneous (wattle) tests for *Bacterium pullorum* infection in poultry breeding stock. Jour. Am. Vet. Med. Assn. 77:47.

———, and Plastridge, W. N.: 1932. Pullorum disease of domestic fowl. Monograph, Storrs Agr. Exper. Sta., Bul. 178-103.

———, and Stoneburn, F. H.: 1909. Bacillary white diarrhea of young chicks. Storrs Agr. Exper. Sta., Bul. 60-29.

———, and Stoneburn, F. H.: 1911. Bacillary white diarrhea of young chicks. (Second report) Storrs Agr. Exper. Sta., Bul. 68:275.

Rhoades, H. E.: 1955. The importance of the stability of some strains of the *Salmonella pullorum* variant in the pullorum agglutination test. Poultry Sci. 34:122.

———, and Alberts, J. O.: 1950. The incidence of serological variants of *Salmonella pullorum* in Illinois. Poultry Sci. 29 579.

Richey, D J.: 1962. Water soluble nitrofuran therapy in pullorum and fowl typhoid in chicks. Am. Jour. Vet. Res. 23:102.

Roberts, E., and Card, L. E.: 1935. Inheritance of resistance to bacterial infection in animals. A genetic study of pullorum disease. Ill. Agr. Exper. Sta., Bul. 419:467.

———, Severens, J. M., and Card, L. E. 1939a. Effect of environment on the expression of resistance and susceptibility to disease in the domestic fowl. Proc. Seventh World's Poultry Cong., p. 451.

———, Severens, J. M., and Card, L. E.: 1939b. Nature of the hereditary factors for resistance and susceptibility to pullorum disease in the domestic fowl. Proc. Seventh World's Poultry Cong., p. 82.

Rose, N. J.: 1962. Personal communication.

Ross, R. T., Holtman, D. F., and Gilliland, R. E.: 1955a. The effect of *Salmonella pullorum* infection on amino acids of the chick. Jour. Bact. 70-272.

———, Holtman, D. F., and Gilliland, R. E.: 1955b. The effect of the introduction of amino acids into chicks infected with *Salmonella pullorum*. Jour. Bact. 70.276.

Roznowska, E. P., and Foltz, V. D.: 1958. Flocculation tests for pullorum disease. Am. Jour. Vet. Res. 19 478.

Runnells, R. A. 1929. Bacillary white diarrhea. Pullorum infection of the domestic fowl. Va. Agr. Exper. Sta., Bul. 265 27.

———, Coon, C. J., Farley, H., and Thorp. F.: 1927. An application of the rapid method agglutination test to the diagnosis of bacillary white diarrhea infection. Jour. Am. Vet. Med. Assn. 70 660.

———, and Van Roekel, H.: 1927a. The occurrence of white diarrhea infection in eggs laid by hens reacting to the agglutination test. Poultry Sci. 6.141.

———, and Van Roekel, H.: 1927b. Further observations on the occurrence of white diarrhea infection in eggs laid by hens reacting to the agglutination test. Poultry Sci. 6.229.

Salmonella Subcommittee of the Nomenclature Committee of the International Society for Microbiology 1954. The genus *Salmonella*, Lignières, 1900. Jour. Hyg. 54:333.

Schaffer, J. M., MacDonald, A. D., Hall, W. J., and Bunyea, H.: 1931. A stained antigen for the rapid whole blood test for pullorum disease. Jour. Am. Vet. Med. Assn. 79 236

Schoenhard, D. E., and Stafseth, H. J.: 1953. Growth curves of *Salmonella pullorum* in different media Jour. Bact. 65 69.

Scholes, J. C.: 1942. Experiments with X-rays on the rules of lymphocytes and body temperatures in the resistance of chicks to *Salmonella pullorum*. Poultry Sci. 21:561.

———, and Hutt, F. B.: 1942. The relationship between body temperature and genetic resistance to *Salmonella pullorum* in the fowl. Cornell Univ. Agr Exper. Sta. Memoir 244:1.

Severens, J. M., Roberts, E., and Card, L. E.: 1945. The effect of sulfonamides in reducing mortality from pullorum disease in the domestic fowl. Poultry Sci. 24:155.

Smith, W. W.: 1954. The treatment of *Salmonella pullorum* in chicks with furazolidone, sulphamerazine, and chloramphenicol. Vet. Record 66.493

Snyser, C. F., and Van Roekel, H.: 1957. The influence of furazolidone on experimental and natural pullorum infection in the chicken. Avian Dis. 1:328.

———, and Van Roekel, H.: 1958. Furazolidone medication in chickens experimentally infected with *Salmonella pullorum*. Avian Dis. 2:428.

Snoeyenbos, G. H., Bachman, B. A., and Van Roekel, H.: 1952. A survey of the incidence of antigenic forms of *Salmonella pullorum* in the United States. Poultry Sci. 31:1009.

Stafseth, H. J., and Corbutt, A. C.: 1940. Identification of *Salmonella pullorum* colonies with immune serum by means of a macroscopic plate test. Am. Jour. Vet. Res. 1:76.

Stokes, J. L., and Bayne, H. G.: 1957. Growth rates of *Salmonella* colonies. Jour. Bact. 74:200.

———, Osborne, W. W., and Bayne, H. G.: 1958. Penetration and growth of *Salmonella* in shell eggs. Food Res. 21:510.

Taylor, J.: 1964. Personal communication.

Tittler, R. P., Heywang, B. W., and Clarke, T. B.: 1928. The occurrence and significance of *Salmonella pullorum* in eggs. Jn. Agr. Exper. Sta., Buf. 255.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflugelzucht. Ferdinand Enke, Stuttgart. P. 104.

Van Roekel, H.: 1931. Eleventh annual report on eradication of pullorum disease in Massachusetts. Mass. Agr. Exper. Sta., Control Series Bul. 58.

———: 1935. A study of variation of *Salmonella pullorum*. Mass. Agr. Exper. Sta. Bul. 319.

———: 1964. Is eradication of pullorum disease realistic? Jour. Am. Vet. Med. Assn. 144:19.

———, and Bullis, K. L.: 1937. Salmonella infections in chickens. Jour. Am. Vet. Med. Assn. 91:48.

———, and Snyser, C. F.: 1962. Unpublished

———, Bullis, K. L., and Snoeyenbos, G. H.: 1947. Twenty-seventh annual report of pullorum disease eradication in Massachusetts. Mass. Agr. Exper. Sta., Control Series Bul. 134.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1932. Twelfth annual report on eradication of pullorum disease in Massachusetts. Mass. Agr. Exper. Sta., Control Series Bul. 63.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1935. Fifteenth annual report on eradication of pullorum disease in Massachusetts. Mass. Agr. Exper. Sta., Control Series Bul. 78.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1937. Maltose fermenting *S. pullorum* strains. Mass. Agr. Exper. Sta., Ann. Rep., Bul. 339.87.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1941. Mass. Exper. Sta., Ann. Rep., Bul. 378.103.

Villani, S.: 1937. Sulla recettività di alcune specie di volatili all'infezione sperimentale da *B. pullorum*. Profilassi, 10(4).148.

Ward, A. R., and Gallagher, B. A.: 1927. An intradermal test for *Bacterium pullorum* infection in fowls. U.S.D.A., Bul. 517

Weldin, J. C., and Weaver, H. J.: 1950. Transmission of pullorum disease from chick to chick. Poultry Sci. 9:176.

W. R. H.: 1928. *New England Conference of Laboratory Workers in Bacillary White Diarrhea* Control. Jour. Am. Vet. Med. Assn. 73 263.

Williams, J. E: 1951. Use of the spot test in the diagnosis of pullorum disease. Poultry Sci 30.125.

———: 1953a. Antigenic studies using ammonium sulfate. I. The relative sedimentation effect of ammonium sulfate on the various antigenic types of *Salmonella pullorum*. Am. Jour. Vet. Res. 14:453

———: 1953b. Antigenic studies using ammonium sulfate. II The macroscopic ammonium sulfate sedimentation test for distinguishing the antigenic forms of *Salmonella pullorum* Am Jour. Vet. Res. 14:465.

———, and Harris, M. J.: 1956. Antigenic studies using ammonium sulfate. IV. The sediment ation effect of ammonium sulfate on *Salmonella gallinarum* Am Jour Vet Res 17 535

———, and MacDonald, A. D.: 1955 The past, present, and future of Salmonella antigens for poultry. Proc. Book Am Vet Med Assn. 333.

———, Pomeroy, B. S., Fensterbacher, R., and Holland, A.: 1949. The incidence of variant pul lorum in Minnesota. Cornell Vet 39 129

Williams, L. P: 1964 Personal communication

Wilson, J. E.: 1955 The use of furazolidone in the treatment of day-old chicks with *S. pullorum*, *S gallinarum*, *S typhi murium*, and *S thompson*. Vet. Record 67.849.

———: 1956. The treatment of carriers of *Salmonella pullorum* and *Salmonella gallinarum* with furazolidone. Vet Record 68-748

Wright, M. L., and Edwards, P R: 1948. The serologic differentiation of *Salmonella pullorum* forms Am Jour Vet Res 9 386

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9

Paratyphoid and Arizona Infections

Paratyphoid Infections

Paratyphoid infections, as the term is used with reference to poultry, denote a large group of acute or chronic bacterial diseases caused by one or more of the normally motile members of the *Salmonella* genus. For purposes of discussion the nonmotile organisms, *Salmonella pullorum* and *Salmonella gallinarum*, causative organisms of pullorum disease and fowl typhoid, respectively, are generally grouped separately from the paratyphoids. Salmonellosis is often used synonymously with "Salmonella infection" as an inclusive term to designate a disease caused by any one or more members of the *Salmonella* genus. *S. pullorum*, *S. gallinarum*, or a paratyphoid organism such as *S. typhi-murium* may independently or together be the cause of salmonellosis. Chronic intestinal carriers of paratyphoid infections are common; however, the disease seldom occurs in the acute, septicemic form except in young fowl or in mature birds under stress conditions such as virus diseases, inadequate diet, or unsanitary environment.

With the rapid expansion of the poultry industry, paratyphoid infections have become one of the most important groups of bacterial diseases affecting poultry, particularly turkeys. Furthermore, domestic poultry constitutes the largest single reservoir of *Salmonella* organisms existing in nature. As this disease recognizes no international boundaries and few host barriers, nationwide programs to eradicate it have not been attempted. Economically, paratyphoid infections are of most concern to commercial hatcherymen and those engaged in domestic poultry raising. Pet store owners, zoological park directors, pigeon and fancy bird raisers, and those interested in wild game are also concerned with the disease. These diseases, as they occur in poultry and poultry products, are also of very significant interest to those engaged in work in the field of public health.

HISTORY

Moore (1895) recorded the first authentic case of paratyphoid infection in domestic poultry in describing an outbreak of infectious enteritis in pigeons due to a bacillus of the hog cholera group. With improvement of culture procedures for the isolation of Salmonella and a better definition of the characteristics of members of the genus, the frequent association of paratyphoid organisms with disease outbreaks in all types of poultry, as well as other animal species and man, was rapidly established. Rettger et al. (1933) first reported on the occurrence of paratyphoid infections in turkey poults in the United States. Pomeroy and Fenstermacher (1939) observed the infection in Minnesota turkeys in 1932.

The numerous contributions of Dr. P. R. Edwards of the United States Public Health Service, and his colleagues, to knowledge on the incidence, distribution, and antigenic typing of paratyphoid and Arizona infections of fowl in the United States will be cited in later sections of this chapter. Dr. Edwards' laboratory has served as a constant reference point for research and disease control scientists interested in Salmonella infections in this country and abroad. Much valuable information, providing a basis for the systematic classification of the paratyphoid infections of poultry, has been contributed by Dr. Edwards and his co-workers since the early 1930's. Furthermore, paratyphoid infections as they occur in poultry have been related to those occurring in other animal species and man.

Knowledge and interest in the field of paratyphoid infections of fowl in the United States have also been advanced by the North Central Regional Poultry Disease Conference, organized in 1950. This conference is composed of research, diagnostic, and regulatory representatives from 12 states. The North Central States Conference has sought to develop and standardize the cultural and serological methods for the study and control of Salmonella infections of poultry.

There has never existed any official national program for the eradication of paratyphoid infections of poultry such as that in operation for the eradication of pullorum disease and fowl typhoid under the National Poultry and National Turkey Improvement Plans (Anon., 1963b). The latter Plans do provide, however, that the official state agency may at its own discretion take paratyphoid infections under consideration in determining the pullorum-typhoid status of a flock. The National Plans maintain a Committee on Salmonellosis and Related Enteric Diseases of poultry which advises on technical matters relating to these infections. The Animal Disease Eradication Division of U.S.D.A. in cooperation with the National Plans has instituted a Salmonella reporting and investigative service dealing with these infections as they occur in poultry flocks in the various states.

ETIOLOGY

The paratyphoid group of bacteria is included in the large family *Enterobacteriaceae* and is composed of approximately 800 serological types belonging to the genus *Salmonella*, each with a specific type designation. The rapidity with which new types have been added to the ever expanding list of paratyphoids is illustrated by the fact that only about 150 types were recognized at the time of the 1952 edition of this book.

Current procedure is to name Salmonella according to the state or province in which they are first isolated. If this name has already been used, the name of the town where the isolation was first made is employed as the basis for nomenclature. This system of naming members of the *Salmonella* genus has resulted in a list of type designations representative of centers of population in every part of the world.

Organisms of the paratyphoid group are defined as serologically related, Gram-negative and nonsporogenic bacilli: 0.4–0.6 by 1–3 micra in usual dimensions but occasionally forming short filaments. They are

normally motile by means of peritrichous flagella, but nonmotile variants are occasionally encountered under natural conditions. Edwards et al. (1946) and Hirsch (1947) reported some strains of Salmonella that appeared to be nonmotile while possessing well-developed flagella and flagellar antigens.

Paratyphoids are facultative anaerobes and can be readily cultivated on initial isolation, from sources other than feces, on simple beef extract and beef infusion agars and broths. Optimum growth temperature is 37° C. When it is desired to obtain large yields of the organisms, media enhanced with serum, dextrose-starch, brain-heart infusion, beef heart infusion (McNeil and Hinshaw, 1951), colloidal sulfur and glycerin (MacDonald, 1947), or cysteine hydrochloride and glycerin (Harris and Williams, 1957) can be used.

Smooth broth cultures after incubation for 24 hours show a thick, homogeneous turbidity with no pellicle and very little sediment. Rough cultures in broth have a heavy, granular sediment and an almost clear supernatant fluid. Felix and Pitt (1935) working with S. typhi were able to produce rough strains by plating broth cultures which had been maintained at room temperature for several months. Stable rough strains were obtained by Béguin and Grabar (1953) through acetone treatment. Rough to smooth transformations of paratyphoid cultures are not easily accomplished. Animal passage of cultures and treatment with guinea pig complement, as cited by Kauffmann (1950), have been used for obtaining smooth cultures. In order to avoid the development of rough cultures, Kauffmann (1950) recommended that media employed for the storage of cultures should contain no carbohydrates, and subculturing should be done as infrequently as possible. Lyophilization is the best method to prevent roughness of cultures.

Typical colonies of paratyphoids on agar culture are round, slightly raised, and glistening with smooth edges. Colonies are generally 1-2 mm. in diameter depending on the degree of dispersion on the plates. Rough (R) colonies may be encountered both among recently isolated strains and those maintained in the laboratory on artificial media. R forms are dull and granular with irregular edges. From a practical standpoint, it is usually assumed that morphologically rough strains of Salmonella do not contain smooth antigens and such cultures cannot be typed serologically or used in the preparation of antigens for the agglutination test. Attempts have been made to distinguish rough cultures by testing cell agglutinability in saline or trypaflavine (Pampana, 1933), or determining color changes in cell masses suspended in Millon's reagent (White, 1929). None of these methods is entirely satisfactory. Study of colonial morphology and observation of growth in broth are perhaps the most practical procedures that can be followed in the laboratory. Kauffmann (1950) cited serological procedures as the only absolute methods to determine if a culture contains normal smooth antigens.

The following properties, as described by Edwards and Ewing (1962), are typical of practically all members of the paratyphoid group:

Dextrose — Fermented with gas
Lactose — Not fermented
Sucrose — Not fermented
Mannitol — Fermented with gas
Maltose — Fermented with gas
Dulcitol — Usually fermented with gas
Salicin — Not fermented
Sorbitol — Fermented with gas
Adonitol — Not fermented
Inositol — Fermented or not fermented
Indol — Not produced
Methyl Red — Positive
Voges-Proskauer — Negative
Simmons' Citrate — Usually utilized
H_2S — Usually positive
Urea — Not hydrolyzed
Gelatin — Rarely liquefied
KCN — Negative
Nitrates — Reduced
Motility — Positive
Decarboxylases

Lysine — Positive
Arginine — Positive, usually delayed
Ornithine — Positive
Malonate — Negative
Phenylalanine deaminase — Negative

Cultures that do not possess the above characteristics may be excluded from the paratyphoid group unless it can be established that they possess antigens of known Salmonella types. It is not presently customary, however, to include certain Arizona strains possessing Salmonella antigens in the paratyphoid group. S. typhi-murium var. copenhagen, a frequent cause of paratyphoid infection of pigeons, occasionally forms no acid and, more frequently, no gas in maltose broth. Consequently this organism is sometimes confused with S. pullorum on initial examination.

Because of the delayed fermentative properties characteristic of certain enteric organisms, such as the Arizona, prolonged incubation of fermentation broths for three weeks or longer is often advantageous. Sealing of the tubes with cork stoppers dipped in hot paraffin will hasten the reactions. Basal carbohydrate broths with Andrade's indicator have been very widely used for many years in the identification and study of Salmonella cultures. Kauffmann (1950) recommended a basal 1 per cent peptone broth with bromthymol blue as an indicator. Bromcresol purple has also been used by some laboratories as an indicator in the study of Salmonella cultures.

Motility of paratyphoid cultures can be readily demonstrated through the use of semisolid medium as described by Edwards and Ewing (1962). This medium is also useful in the separation of flagellar phases for the preparation of antigens or typing sera.

RESISTANCE AND VIABILITY OF PARATYPHOID ORGANISMS

Paratyphoid organisms are quite susceptible to heat and the majority of the common disinfectants. Most members of the group, suspended in saline, are de-

stroyed by a temperature of 60° C. in approximately 15 minutes. Bierer and Barnett (1961) demonstrated that washing eggs, the shells of which were contaminated with S. typhi-murium, for either 3 minutes or 1 minute at 65° C. resulted in 99.5–100 per cent kill of the organisms. Cresylic acid and lye are frequently employed in the disinfection of poultry premises. Formaldehyde is also widely used as a disinfectant, particularly as a fumigant for incubators and hatchery rooms. Lancaster et al. (1952) reported that S. thompson and S. typhi-murium were more resistant than S. pullorum to the effects of several disinfecting solutions studied.

Hashimoto (1961) found S. senftenberg, in contrast to S. pullorum, to be resistant to the bactericidal properties of egg albumen, yolk, and various embryonic fluids. Watanabe et al. (1959b) demonstrated that embryonic fluid and serum had no bactericidal effect on S. senftenberg. Anellis et al. (1954) reported that in egg albumen Salmonella organisms were more rapidly destroyed by heat at a high pH, and Banwart and Ayres (1957) found that raising the pH of egg albumen to 9 or 10 caused a reduction in the number of surviving Salmonella organisms during processing of the product. Lerche (1957) noted that the addition of 0.25–0.5 per cent of ammonia was necessary to destroy Salmonella in egg white. Simskaya (1955) demonstrated that the inactivation of amylase in duck eggs can be used as an index or confirm the destruction of Salmonella organisms in such eggs following heat treatment.

Watts and Wall (1952) found that S. typhi-murium could survive for at least 119 days in ponds in Australia. Kraus and Weber (1958) demonstrated that Salmonella organisms could survive from several weeks to 3 months in drinking water and natural surface water, being affected independently by the nutritional conditions and the temperature of the water. Adler et al. (1953) were able to isolate S. typhi-murium from litter 44 days after experimental infection of poults which were allowed to run on the litter. Felsenfeld and

Young (1945) demonstrated that Salmonella could survive for several weeks on vegetables kept at room temperature. Steiniger (1961), in the examination of 100,000 samples of birds' feces found in nature, reported that Salmonella were more frequently isolated from feces found on vegetation than on stones and soil. Salmonella survived for 28 months on plant material allowed to dry slowly. It was concluded that fodder grown in fields sprayed with sewage effluent should not be mixed with concentrates that would favor the multiplication of Salmonella.

Mair and Ross (1960) reported that *S. typhi-murium* was found to survive in urban garden soil in England for at least 280 days, the extent of their study. Slavkov (1961) found that Salmonella organisms survived in soil for 120–150 days depending on pH, temperature, and the presence of nutrients or inhibitors. Price *et al.* (1962) cited information to indicate that Salmonella organisms may remain viable in duck feces for 28 weeks. Malathion in fly sprays was not found to kill the organisms in feces. Sylwester (1961) reported that *S. typhi-murium* remained viable in dried pupae of Calliphora flies for a period of one year.

Pomeroy and Fenstermacher (1939) found that paratyphoid organisms survived in turkey eggshells at incubator temperature for at least 11 months; in feces at incubator temperature from 77 days to 11 months; on eggshells at 50° F. from 191 to 346 days; on eggshells exposed to the varying conditions of the elements for 135 to 350 days depending upon the organism. The same investigators (1941) demonstrated that *S. typhi-murium* could survive in the contents of turkey eggs at incubator temperature for a period of at least 13 months. Buxton and Gordon (1947) found that *S. thompson* could survive on the surface of chicken eggs for at least 21 days under ordinary conditions of storage at room temperature. Gregory (1948), using an incubator at a temperature of 100° F. and a wet bulb reading of 82°–86° F., found that the shell surface of 2 of 26 turkey eggs contaminated with *S. typhi-murium* remained infected after 28 days.

Huey and Edwards (1958) found that 9 per cent of *S. typhi-murium* strains isolated from poultry after 1956 were resistant to tetracyclines when compared with other cultures isolated prior to 1948. They attributed this to the use of antibiotics in poultry feeds. In further studies, Ramsey and Edwards (1961) found that 29 of 100 *S. typhi-murium* cultures isolated from fowls in 1959 and 1960 were resistant to the tetracyclines. Garside *et al.* (1960) demonstrated a tenfold increase in resistance to chlortetracycline in the case of a single colony inoculum of *S. typhi-murium* administered to chicks receiving the antibiotic in their diet. The organisms were capable of resisting 210 p.p.m. of chlortetracycline after 4 passages. Subsequent passage of the resistant cultures through chicks receiving no drug in their feed revealed that resistance declined gradually, but at the end of 14 weeks some strains were still 4 times as tolerant to the antibiotic as the normal strains. Hobbs *et al.* (1960) found that a strain of *S. typhi-murium* resistant to chlortetracycline grew more rapidly than spoilage organisms at 22° C. on the skin of dressed poultry that had been immersed in slush ice containing 10 p.p.m. chlortetracycline.

Lancaster and Crabb (1953a) reported that *S. typhi-murium* and *S. thompson* rapidly lose their viability on the shell of whole chicken eggs maintained under normal incubator temperature. At room temperature the organisms were found to survive for approximately 21 days on clean eggs and for a longer period on artificially dirty eggs. Increased humidity prolonged the viability of the organisms. Watanabe *et al.* (1959a) found that *S. senftenberg* survived for 10 days on the surface of the eggshell at room temperature. In the incubator the organism penetrated the shell in 4 to 8 days and was found to penetrate more rapidly through eggshells that were filed. Embryos which were invaded by *S. senftenberg* through the shell stopped their development and could not hatch.

NATURAL DISTRIBUTION, INCIDENCE, AND ECONOMIC IMPORTANCE

Paratyphoid infections of poultry exist in all parts of the world; however, it has often been found that particular serological types are more frequently encountered in one region than in another. Wilson (1948) called attention to the occurrence of 24 new Salmonella types encountered in human cases of food poisoning in Great Britain. Six of these types were commonly found in poultry in the United States; however, they had not been found in Great Britain prior to the large scale importation of egg powder during World War II.

Certain Salmonella types have become widespread in a country or an area for a given period of time, and then decreased in incidence to a point of little importance. Gordon and Buxton (1946) noted that S. thompson was first encountered in poultry in Great Britain in 1943, and subsequently ceased to be a problem. Buxton (1957a) stated that S. oranienburg, S. bareilly, and S. montevideo are more frequently isolated from poultry in the United States than in European countries, while S. enteritidis occurs more frequently among poultry in European South countries than it does in North and South America.

Extensive surveys have been conducted to determine the incidence of the various serological types of Salmonella existing in poultry in the United States. Edwards (1939) listed 16 types of Salmonella recovered over a 5-year period from 100 separate outbreaks among poultry. Bruner and Edwards (1941) examined 900 cultures serologically and found 64 of this number were members of the paratyphoid group. Ninety-two per cent of these cultures were isolated from fatal infections of fowl. Edwards and Bruner (1943) examined several thousand cultures of Salmonella obtained from many areas in the United States. They did not subdivide the fowl into their respective species; however, 42 Salmonella types were found in poultry. Pomeroy and Fenstermacher (1941) recorded the isolation of 20 Salmonella types

from turkey poults in Minnesota. Pomeroy (1944) listed 24 Salmonella types recovered from poults in Minnesota. S. typhi-murium was found in approximately 75 per cent of the paratyphoid outbreaks. Fenstermacher (1952) listed 40 types that had been isolated from turkeys in the Minnesota laboratory.

Hinshaw et al. (1944) listed a total of 48 Salmonella types isolated from poultry in the United States. Edwards et al. (1948a, 1948b) examined 12,831 cultures of Salmonella that had been isolated during the period 1934–47 from 47 animal species (including fowl, reptiles, lower mammals, and man) and from other sources.

Edwards et al. (1948c) reported on the serological analysis of 6,587 Salmonella cultures isolated from fowl. Practically all of the cultures were derived from acute, fatal infections of young birds. A small number were isolated from adult birds affected with acute infections and many others from the intestines of adult chickens that were apparently normal carriers. Fifty-eight different Salmonella types, exclusive of S. pullorum and S. gallinarum, were recovered from fowl. S. typhi-murium was encountered most frequently producing 30.8 per cent of the outbreaks and composing 37.7 per cent of the cultures. It was concluded that any Salmonella type other than those types showing strict host adaptation, such as S. typhi and S. abortus-equi, may under proper circumstances cause highly destructive diseases in poultry. No correlation was found between the type of Salmonella isolated and the severity of the disease; however, it was felt that such types as S. typhi-murium, S. oranienburg, S. montevideo, S. bareilly, and S. newport usually produced the highest percentages of mortality.

Perelli-Minetti et al. (1948) reported the isolation of 28 types of paratyphoid from chickens and turkeys over a period of 6½ years in the poultry diagnostic laboratories of California. Of a total of 167 turkey isolations, 58.6 per cent were types other than S. typhi-murium. Of 12 cases in adults, 91.6 per cent were S. typhi-mu-

rium. Smith (1959) cultured the intestinal walls of 280 normal chickens in Essex, England, and was unable to isolate *Salmonella* from any of the samples.

Lukas and Bradford (1954) reported that paratyphoid infections were responsible for approximately 21 per cent of the disease problems with which the turkey growers in the Turlock area of California were concerned during the first 6 months of 1952. A total of 241 cultures of paratyphoid organisms, representing 30 serological types, was isolated from turkey poults on routine necropsy. *S. typhi-murium* accounted for 46.5 per cent of the uncomplicated paratyphoid outbreaks. Pomeroy *et al.* (1957a) found that 15 per cent of the consignments of poults received in the Minnesota laboratory in 1956 were infected with paratyphoid.

Moran (1959a) cited typing data which indicated that *S. typhi-murium* was encountered four times as frequently in turkeys as in chickens in the United States in 1957 *S. heidelberg* and *S. infantis*, which had not been reported in fowl in the United States in the earlier report of Edwards *et al.* (1948c), comprised 8 per cent and 6.7 per cent, respectively, of the cultures from chickens. Moran (1960) found that of 1,178 *Salmonella* cultures typed from animals during 1958, 87.2 per cent were from avian sources. Sixty-nine types were identified, 40 of which occurred in turkeys and 32 in chickens. Moran (1961b) reported the isolation of 57 types of *Salmonella* from turkeys and 52 types from chickens in the United States during a survey period of 4½ years (1957–1961). *S. typhi-murium* was by far the most common and accounted for 22 per cent of all *Salmonella* types encountered in animals. *S. typhi-murium* cultures isolated from turkeys outnumbered those from chickens by about 2 to 1.

A total of 117 paratyphoid types that have been isolated from chickens and/or turkeys in the United States are listed in Table 9.1. Fifteen new types not previously reported from poultry in the United States have been added to the list since the

fourth edition of this book. The information included in the table has been developed from published reports and from data supplied by diagnostic laboratories, typing centers, and research workers in various parts of the country. While the listings may be incomplete, it is considered that most types that have been reported prior to 1964 from chickens and turkeys are represented. As additional laboratories are encouraged to submit cultures for typing, the listings will undoubtedly be considerably increased. Some of the types listed in Table 9.1 have not been associated with disease outbreaks, but were recovered from the intestines of birds that were apparently normal carriers. However, most of the cultures were derived from acute, fatal infections in young chicks or poults, and were isolated from the internal organs or intestinal contents. Isolations from unabsorbed yolks, ovarian cysts, and oviducts represent only a small percentage of the culture types listed. Original references to the description of most of the *Salmonella* types included in Table 9.1 are listed by Kauffmann (1954).

Buxton (1957a) in a comprehensive review of salmonellosis in poultry, presented a worldwide survey of *Salmonella* types occurring in poultry. A total of 90 types of paratyphoid organisms was reported from 12 species of fowl. Many of these serotypes were reported to have caused only a few minor epizootics and some to have been isolated only from apparently healthy birds. The reader seeking reference material on the host species, origin, and distribution of *Salmonella* types occurring in poultry in various parts of the world is referred to this excellent review.

Some of the *Salmonella* types isolated from poultry in other parts of the world that have not yet been reported from fowls in the United States include *S. bonariensis, S. brancaster, S. brandenburg, S. chicago, S. goettingen, S. sturi, S. lille, S. mbandaka, S. ness-ziona, S. oslo, S. schleissheim,* and *S. weybridge.*

Lerche (1939) reported the following types have been isolated from poultry in

TABLE 9.1

LIST OF PARATYPHOIDS ISOLATED FROM TURKEYS AND/OR CHICKENS
IN THE UNITED STATES

S. aberdeen	S. duesseldorf	S. london	S. rutgers
S. alachua	S. eastbourne	S. madelia	S. saint-paul
S. albany	S. edinburg	S. manchester	S. san-diego
S. amager	S. enteritidis	S. manhattan	S. san-juan
S. amersfoort	S. essen	S. manila	S. schwarzengrund
S. amherstiana	S. florida	S. meleagridis	S. senftenberg
S. anatum	S. fresno	S. menston	S. siegburg
S. banana	S. gaminara	S. mgulani	S. simbury
S. bareilly	S. give	S. minneapolis	S. stanley
S. berkeley	S. grumpensis	S. minnesota	S. takoradi
S. berta	S. hamilton	S. mission	S. takony
S. binza	S. harrisonburg	S. montevideo	S. tallahassee
S. blockley	S. hartford	S. muenchen (oregon)	S. tel-aviv
S. bovis-morbificans	S. heidelberg	S. muenster	S. tennessee
S. braenderup	S. hvittingfoss	S. new-brunswick	S. thomasville
S. bredeney	S. illinois	S. new-haw	S. thompson
S. budapest	S. indiana	S. newington	S. typhi
S. california	S. infantis	S. newport (puers)	S. typhi-murium
S. cambridge	S. irumu	S. norwich	S. typhi-murium
S. canoga	S. israel	S. onderstepoort	(var. copenhagen)
S. cerra	S. java	S. oranienburg	S. uganda
S. champaign	S. javiana	S. orian	S. uno
S. chester	S. johannesburg	S. panama (italiana)	S. urbana
S. cholerae-suis	S. kaapstad	S. paratyphi B	S. vejle
S. eanard	S. kentucky	(schottmülleri)	S. westhampton
S. corvallis	S. kingstan	S. pensacola	S. wichita
S. cubana	S. lexington	S. pamona	S. worcester
S. denver	S. litchfield	S. paona	S. warthington
S. derby	S. livingstone	S. reading	S. zanzibar
S. dublin	S. lumia	S. rubislaw	

Germany: *S. typhi-murium, S. enteritidis* Gaertner (vars. Essen and Kiel), *S. cholerae-suis, S. newport, S. senftenberg, S. oranienburg,* and *S. anatum.* Sedlmeier et al. (1957) found 46 different Salmonella types among chickens in Germany. These were described as human-pathogenic types. Hygienic measures necessary to control or eliminate these infections were described. Feils (1957) reported the identification of 300 cultures of Salmonella from poultry in Germany. *S. bredeney, S. bareilly, S. infantis, S. binza, S. typhi-murium,* and *S. anatum* were among the types isolated. Possible importation of these organisms in protein animal feeds was mentioned, and the public health significance of these infections was emphasized. Hansen (1942), in Scandinavia, studied 308 cultures isolated from poultry, 303 of which were *S. typhi-murium,* 3 *S. enteritidis* (var. Essen), and 2 were *S. dublin.* Henning (1939), following a study of salmonellosis in South Africa, reported that *S. typhi-murium, S.*

anatum, S. amersfoort, and S. typhi were recovered from poultry.

Kampelmacher (1963) reported that with the exception of ducks, poultry surprisingly has not been found so far to constitute an important reservoir of Salmonella organisms in the Netherlands. Marthedal (1962) noted the marked increase of Salmonella infections in poultry in Denmark since 1944. The large number of new types found have been ascribed mainly to imported meat and bone meal that has not been sufficiently heated. Das et al. (1959) stated that the incidence of avian salmonellosis is increasing in India. Perek and Rabinovitz (1957) reported that 12 Salmonella types have been isolated from poultry in Israel.

Atkinson (1956) reviewed the serological typing of 3,340 Salmonella cultures in Australia during the period 1944–1954. Fifty-nine different Salmonella types were found with *S. typhi-murium* and *S. bovis-morbificans* being the most common types iso-

lated from both humans and animals.

Gordon and Buxton (1946), in a study of avian salmonellosis in Great Britain, found during the period 1933–44 that of a total of 6,578 groups of young birds examined, 4.1 per cent were infected with Salmonella organisms other than *S. pullorum* and *S. gallinarum*. The types of paratyphoid organisms isolated were *S. typhi-murium*, *S. thompson*, *S. enteritidis*, *S. california*, *S. bareilly*, *S. montevideo*, and *S. anatum*. Gordon (1959) reported that up to 1959, 50 types of Salmonella have been isolated from poultry in Great Britain. Several types not previously reported have been isolated from chickens; however, these new types have not tended to become established in poultry flocks and outbreaks due to them are restricted.

Bigland et al. (1962) reviewing Salmonella isolations from Alberta, Canada, for the years 1949 to 1960, reported a total of 22 types of paratyphoid, with 1,227 of a total of 1,242 isolations from avian species being made from turkeys and chickens. *S. heidelberg*, first isolated in Alberta in 1952, has been the most commonly isolated Salmonella type of avian origin for several years. Sakazaki et al. (1959) examined a total of 2,482 Salmonella and 16 Arizona cultures isolated from man and animals in Japan from 1949 to 1957. Fifty-eight types of Salmonella and 9 types of Arizona were identified. *S. senftenberg* was the most frequent Salmonella type isolated from fowl in contrast to the situation in the United States. Three Salmonella types which seem to be exclusive for Japan were repeatedly isolated. Arizona strains were cited as rare in Japan, and none of those isolated came from poultry.

The epizootiology of Salmonella infections among individual flocks is often complex due to the wide distribution of the organisms in fowl and the practice of bringing the eggs from different flocks together in one hatchery. Edwards and Bruner (1940) described an extensive study of multiple types of paratyphoids in individual flocks. Pomeroy and Fenstermacher (1941) reported that from one farm where poults had been accumulated from several sources over a period of 3 years, *S. typhimurium*, *S. derby*, *S. give*, *S. oranienburg*, *S. senftenberg*, and *S. anatum* were isolated. From the same farm in previous years other Salmonella types had been isolated. Hinshaw et al. (1944) also reported multiple types of salmonellosis on the same ranch.

Edwards et al. (1948a) found more than one Salmonella type existing in the same flock in 165 instances. Akiyama et al. (1959) isolated 4 Salmonella types from a group of 7-day-old chicks that were also found to be infected with *S. pullorum*. It was assumed that the organisms infected the eggs during incubation. Ballantyne (1953) reported the isolation from one turkey farm of 4 different serological types of Salmonella. Boyer et al. (1962) reported multiple Salmonella types in several individual cases of salmonellosis in both chicks and poults. It was noted that simultaneous infections are not unusual.

The isolation of 2 or more Salmonella types from a single bird was reported by Edwards et al. (1948a, b) in 51 cases of avian paratyphoid infections. Four Salmonella types were recovered from the liver of one poult, and 3 from the liver of a second.

From an economic viewpoint paratyphoid infections are among the most important bacterial diseases of the hatching industry and result in high death losses among all types of young poultry. The occurrence of this disease in valuable breeding stock is extremely costly. Because of its chronic nature and difficulty of eradication it is capable of terminating breeding operations in which large amounts of money may have been invested. Fertility, hatchability, and egg production may be seriously impaired (Graham and Michael, 1936; Pomeroy and Fenstermacher, 1941). The disease has a definite stunting effect on surviving birds and a debilitating influence on poultry of all ages increasing their susceptibility to many diseases.

SEROLOGY OF THE PARATYPHOID GROUP

Paratyphoid organisms have been subjected to detailed antigenic study. The approximately 800 serological types presently recognized are listed in the Kauffmann-White diagnostic schema. Most Salmonella organisms possess both somatic (O) and flagellar (H) antigens. The O antigen is associated with the bacterial cell proper and is resistant to both alcohol and heat treatment. The H antigen is a part of the flagella and is both alcohol- and heat-labile. The various O antigenic factors are designated with arabic numerals while the H antigenic factors are divided into phases 1 and 2, which are designated with small letters and arabic numerals, respectively. Thus, the complete antigenic formula of S. typhimurium is 1, 4, 5, 12 (O): i, 1, 2 (H). Marthedal (1962) reported a variation in the somatic antigens of cultures derived from 320 outbreaks of S. typhimurium infection in poultry in Denmark. Factors 4, 5, and 12 were easily demonstrated in most cultures; however, factor 1 was variable.

Highly specialized serological procedures have been developed for the antigenic analysis and classification of Salmonella cultures. In conducting the antigenic analyses, sera containing agglutinins for specific antigenic factors are used in macroscopic plate and tube agglutination tests to determine, first, the O and, subsequently, the H antigenic structure of each culture. After the O and H antigenic factors of a culture have been determined its identification merely requires reference to the Kauffmann-White schema. The schema includes 10 main serological groups, with groups C and E possessing additional subgroups.

Most poultry diagnostic laboratories do not find it practical to engage in the serological analysis of Salmonella cultures, but rather rely upon the U.S.D.A.'s National Animal Disease Laboratory, Ames, Iowa; the State Health Department Laboratory; or the Communicable Disease Center's Enteric Bacteriology Laboratory, Atlanta, Georgia, for this service. It is strongly recommended that all Salmonella cultures isolated from poultry be sent to Diagnostic Services, National Animal Disease Laboratory, Box 70, Ames, Iowa, for typing. This will permit cultures of avian origin to be examined at one central laboratory for better correlation, review, and distribution of results as accumulated. Thus, accurate, current information on the incidence and distribution of Salmonella types among poultry will be available to guide the formulation of control programs for those found to be of most importance.

Polyvalent sera for examination of Salmonella and Arizona cultures are very useful in preliminary culture screening. These sera are generally employed in rapid slide tests in a suitable dilution, and agglutination is easily read. Some are available commercially. Kauffmann and Edwards (1947, 1957) described simplified methods for the serological identification of the most important Salmonella types. Edwards et al. (1948c) found that 99.5 per cent of all Salmonella types from fowls that they examined belonged to groups B, C, D, and E or were S. worthington or S. minnesota. This prompted them to suggest that a polyvalent serum covering the somatic and frequently occurring flagellar antigens of the above four groups and S. worthington and S. minnesota would be very helpful in the diagnosis and study of avian salmonellosis. Bruner (1957) described the preparation of a polyvalent Salmonella typing serum by injecting rabbits with a mixed formolized broth antigen representative of the known components of the Salmonella antigenic mosaic.

There is marked variation in the somatic and flagellar antigenic structure of Salmonella types infecting poultry. The antigenic formula of each of 117 types of paratyphoids isolated from turkeys and chickens in the United States is listed in Table 9.2. Organisms representative of all

TABLE 9.2

ANTIGENIC GROUPING OF PARATYPHOIDS ISOLATED FROM TURKEYS AND/OR CHICKENS IN THE UNITED STATES

Group	Type	O-Antigen	H-Antigen Phase 1	H-Antigen Phase 2	Group	Type	O-Antigen	H-Antigen Phase 1	H-Antigen Phase 2
A	....				D	S. fresno	(9),46	z_{10}	....
B	S. banana	4,5,12	m,t	....		S. israel	9,12	e,h	1,5
	S. bredeny	4,12,27	i	1,7		S. jamesa	1,9,12	z_{4},z_{24}	....
	S. budapest	1,4,12	g,t	....		S. panama	1,9,12	l,v	1,5
	S. california	4,12	g,m,t	....		S. pensacola	9,12	m,t	....
	S. chester	4,5,12	e,h	e,n,x		S. typhi	9,12,Vi	d	....
	S. derby	1,4(5),12	f,g	....	E_1	S. amager	3,10	y	1,2
	S. eisen	4,12	g,m	....		S. anatum	3,10	e,h	1,6
	S. heidelberg	4,5,12	r	1,2		S. give	3,10	l,v	1,7
	S. indiana	4,12	z	1,7		S. lexington	3,10	z_{10}	1,5
	S. iowa	1,4,5,12	b	(1,2)		S. london	3,10	l,v	1,6
	S. kaapstad	4,12	e,h	1,7		S. meleagridis	3,10	e,h	1,w
	S. kingston	1,4,12,27	g,s,t	....		S. muenster	3,10	e,h	1,5
	S. paratyphi B	1,4,5,12	b	1,2		S. orion	3,10	y	1,5
	S. reading	4,5,12	e,h	1,5		S. rutgers	3,10	z_{10}	1,7
	S. saint-paul	1,4,5,12	e,h	1,2		S. uganda	3,10	l,z_{13}	1,5
	S. san-diego	4,5,12	e,h	e,n,x		S. vejle	3,10	e,h	1,2
	S. schwarzengrund	1,4,12,27	d	1,7		S. westhampton	3,10	g,s,t	....
	S. stanley	4,5,12	d	1,2		S. zanzibar	3,10	k	1,5
	S. typhi-murium	1,4,5,12	i	1,2	E_2	S. binza	3,15	y	1,5
	S. typhi-murium (var. copenhagen)	1,4,12	i	1,2		S. cambridge	3,15	e,h	1,w
						S. hamilton	3,15	z_{10}	....
C_1	S. amersfoort	6,7	d	e,n,x		S. manila	3,15	z_{10}	1,5
	S. bareilly	6,7	y	1,5		S. new-brunswick	3,15	l,v	1,7
	S. braenderup	6,7	e,h	1,5		S. new-haw	3,15	e,h	1,5
	S. cholerae-suis	6,7	c	1,5		S. newington	3,15	e,h	1,6
	S. concord	6,7	l,v	1,2	E_3	S. canepa	(3),(15),34	g,s,t	....
	S. denver	6,7	a	e,n,x		S. harrisonburg	(3),(15),34	z_{10}	1,6
	S. edinburg	6,7	b	1,5		S. illinois	(3),(15),34	z_{10}	1,5
	S. hartford	6,7	y	e,n,x		S. minneapolis	(3),(15),34	e,h	1,6
	S. infantis	6,7	r	1,5		S. thomasville	(3),(15),34	y	1,5
	S. irumu	6,7	r	1,5	E_4	S. senftenberg	1,3,19	g,s,t	....
	S. livingstone	6,7	l,v	1,5		S. simsbury	1,3,19	z	....
	S. lomita	6,7	e,h	1,w		S. taksony	1,3,19	i	z_{6}
	S. menston	6,7	g,s,t	....	F	S. aberdeen	11	i	1,2
	S. mission	6,7	d	1,5		S. rubislaw	11	r	e,n,x
	S. montevideo	6,7	g,m,s	....	G	S. cubana	1,13,23	l,z_{13}	....
	S. noru ich	6,7	e,h	1,6		S. grumpensis	13,23	d	1,7
	S. oranienburg	6,7	m,t	....		S. poona	13,22	z	1,6
	S. sanjuan	6,7	a	1,5		S. luschta	1,13,23	d	z_{6}
	S. tennessee	6,7	z	1,5		S. worcester	1,13,23	m,t	e,n,x
	S. thompson	6,7	k	1,5		S. worthington	1,13,23	l,w	z
C_2	S. albany	(8)*,20	z_{4},z_{24}	....	H	S. florida	(1),6,14,25	d	1,7
	S. amherstiana	(8)	l,(v)	1,6		S. madelia	(1),6,14,25	d	....
	S. blockley	6,8	z	e,n,x		S. onderstepoort	(1),6,14,25	z_{4},z_{23}	....
	S. bovis-morbificans	6,8	r	1,5		S. siegburg	6,14,18	z_{4},z_{23}	....
	S. corvallis	(8),20	z_{4},z_{23}	....	I	S. gaminara	16	d	1,7
	S. duesseldorf	6,8	z_{4},z_{24}	....		S. hvittingfoss	16	b	e,n,x
	S. kentucky	(8),20	i	z_{6}	Further groups	S. alachua	35	z_{4},z_{24}	....
	S. litchfield	6,8	l,v	1,2		S. berkeley	43	i	1,5
	S. manchester	6,8	l,v	1,7		S. cerro	18	z_{4},z_{23}	....
	S. manhattan	6,8	d	1,5		S. champaign	39	k	1,5
	S. muenchen	6,8	d	1,2		S. johannesburg	1,40	b	e,n,x
	S. newport	6,8	e,h	1,2		S. mgulani	38		....
	S. takoradi	6,8	i	1,5		S. minnesota	21	b	e,n,x
	S. tallahassee	6,8	z_{4},z_{24}	....		S. pomona	28	y	1,7
	S. una	6,8	z	1,5		S. tel-aviv	28	k	e,n,x
D	S. berta	9,12	f,g,t	....		S. wihana	30	y	e,n,x
	S. dublin	1,9,12	g,p	....					
	S. eastbourne	1,9,12	e,h	1,5					
	S. enteritidis	1,9,12	g,m	....					

*Indicates that this antigen may be absent.

the major antigenic groups in the Kauff-mann-White schema, with the exception of serological group A, have been isolated from chickens and turkeys.

Approximately 80 per cent of all Salmonella types isolated from turkeys and chickens in the United States are members of antigenic groups B, C, D, and E. The greatest number of paratyphoid types belong to group C followed by groups E, B, and D in sequence. Because of the very frequent occurrence of S. typhi-murium in paratyphoid outbreaks in turkeys in the United States, the greatest number of cultures isolated is representative of serological group B.

As illustrated in Table 9.2, organisms within a given serological group share common somatic antigens. Flagellar antigens are also shared within and between groups. An example of the existence of common somatic antigenic factors in two separate groups of the Kauffmann-White schema is illustrated by organisms in groups B and D, which both possess antigen 12. This common somatic antigen accounts for the fact that chickens and turkeys infected with S. typhi-murium (1, 4, 5, 12) or some other member of group B, may be detected when tested with S. pullorum (9, 12) antigen. As would be expected, the serum titer of birds infected with S. typhi-murium is lower when tested with pullorum antigen than with a homologous antigen.

Hinshaw and McNeil (1943a) found that the 1-25 dilution tube agglutination test for pullorum disease in turkeys will detect about 25 per cent of the S. typhi-murium reactors because of the cross agglutination in antigen 12. Bierer and Vickers (1960a) found the pullorum whole-blood and tube agglutination tests to be of little value in detecting birds infected with S. typhi-murium. Burr et al. (1957) found that approximately 50 per cent of a group of 13 chickens, artificially infected with S. heidelberg (4, 5, 12), revealed no titer to S. pullorum tube agglutination antigen at a 1-25 dilution, but gave at least a 2+ reaction at the same dilution with S. heidelberg antigen. Chang et al. (1957) found

that removal of the Bursa of Fabricius in young chickens reduced antibody production following inoculation of S. typhi-murium antigen.

Bahr and Christensen (1933), Van Roekel and Bullis (1937), Pomeroy and Fenstermacher (1944), Hinshaw and McNeil (1944a), and Becker (1957) have discussed the difficulties that are encountered in testing chickens and turkeys for the control of pullorum disease when certain types of paratyphoid infections are present in flocks. Wilson (1947) reported the isolation of S. typhi-murium from two chickens that reacted with pullorum antigen.

PATHOGENICITY

Most death losses from paratyphoid infections of poultry are encountered during the first two weeks after hatching with the highest losses occurring between the sixth and tenth day. The infection seldom causes severe mortality in birds more than one month old. Pigeons, parakeets, and canaries may be cited as exceptions, for the disease does occur more frequently in the acute form in adults of these species.

Mortality rates among broods of young birds under natural conditions usually vary from negligible to 10 or 20 per cent; however, mortality rates of 80 per cent or higher are encountered in severe outbreaks. The pathogenic properties of Salmonella are due to endotoxins which are closely associated with the somatic portion of the organism. Buxton (1958) called attention to the fact that nutrition may have a significant effect on the susceptibility of animals to Salmonella infections and may be associated with factors concerning the development of immunity in the host and alterations in the virulence of the infecting organism.

Adult birds infected with paratyphoid organisms generally show no outward symptoms; however, they may serve as intestinal carriers of the infection over long periods of time. Paratyphoid infections exhibit little or no selectivity in their pathogenicity for specific strains or breeds of birds.

TABLE 9.2

ANTIGENIC GROUPING OF PARATYPHOIDS ISOLATED FROM TURKEYS AND/OR CHICKENS IN THE UNITED STATES

Group	Type	O-Antigen	H-Antigen Phase 1	Phase 2
A	....			
B	S. banana	4,5,12	m,t	....
	S. breedney	1,4,12,27	m,t	...
	S. budapest	1,4,12	g,t	...
	S. california	4,12	g,m,t	....
	S. chester	4,5,12		e,n,x
	S. derby	1,4,(5),12	f,g	...
	S. essen	4,12	g,m	...
	S. heidelberg	4,5,12	r	1,2
	S. indiana	4,12	z	1,7
	S. java	1,4,5,12	b	(1,2)
	S. kaapstad	4,12	e,h	1,7
	S. kingston	1,4,12,27	g,s,t	
	S. paratyphi B	1,4,5,12	b	1,2
	S. reading	4,5,12	e,h	1,5
	S. saint-paul	1,4,5,12	e,h	1,2
	S. san-diego	4,5,12	e,h	1,5
	S. schwarzengrund	1,4,12,27	d	e,n,x
	S. stanley	4,5,12	d	1,2
	S. typhi-murium	1,4,5,12	i	1,2
	S. typhi-murium (var. copenhagen)	1,4,12	i	1,2
C₁	S. amersfoort	6,7	d	1,7
	S. bareilly	6,7	y	1,5
	S. beerenverp	6,7	e,h	1,5
	S. cholerae-suis	6,7	c	1,5
	S. concord	6,7	l,v	1,2
	S. denver	6,7		e,n,z₁₅
	S. edinburg	6,7	b	1,5
	S. hartford	6,7		e,n,x
	S. infantis	6,7	r	1,5
	S. irumu	6,7	l,v	1,5
	S. livingstone	6,7	d	1,w
	S. lomita	6,7	e,h	1,5
	S. meleton	6,7	g,s,t	
	S. mission	6,7	z	1,5
	S. montevideo	6,7	g,m,s	
	S. norwich	6,7	e	1,6
	S. oranienburg	6,7	m,t	
	S. sanjuan	6,7	a	1,5
	S. tennessee	6,7		z₂₉
	S. thompson	6,7	k	1,5
C₂	S. albany	(8)*,20	z₄,z₂₄	
	S. amherstiana	(8)	l,(v)	
	S. blockley	6,8	k	1,6
	S. bous-morbificans	6,8	r	1,5
	S. corvallis	(8),20	z₄,z₂₃	
	S. duesseldorf	6,8	z₄,z₂₄	
	S. kentucky	(8),20	z₄,z₂₃	
	S. litchfield	6,8	z₁₀	
	S. manchester	6,8	l,v	1,7
	S. manhattan	6,8	d	1,5
	S. muenchen	6,8	d	1,2
	S. newport	6,8	e,h	1,2
	S. takoradi	6,8	i	1,5
	S. tallahassee	6,8	z₄,z₂₃	
	S. uno	6,8	z₄,z₂₄	
D	S. berta	9,12	f,g	..
	S. dublin	1,9,12	g,p	
	S. eastbourne	1,9,12	e,h	
	S. enteritidis	1,9,12	g,m	1,5

Group	Type	O-Antigen	H-Antigen Phase 1	Phase 2
D	S. feesto	(9),46	z₁₀	e,n,x,z₁₅
	S. israel	9,12	e,h	e,n,z₁₅
	S. jaticana	1,9,12	l,v	1,5
	S. panama	1,9,12	l,v	1,5
	S. pensicola	9,12	y	1,5
	S. typhi	9,12,Vi	d	...
E₁	S. amager	3,10	y	1,2
	S. anatum	3,10	e,h	1,6
	S. give	3,10	l,v	1,7
	S. lexington	3,10	z₁₀	1,5
	S. london	3,10	l,v	1,6
	S. meleagridis	3,10	e,h	1,w
	S. muenster	3,10	e,h	1,5
	S. orion	3,10	y	1,5
	S. rutgers	3,10	l,z₂₈	1,7
	S. uganda	3,10	l,z₁₃	1,5
	S. tefe	3,10	l,v	1,2
	S. westhampton	3,10	g,s,t	..
	S. zanzibar	3,10	k	1,5
E₂	S. binza	3,15	y	1,5
	S. cambridge	3,15	e,h	1,w
	S. hamilton	3,15	z₁₀	
	S. manila	3,15	z₁₀	1,5
	S. new-brunswick	3,15	l,v	1,7
	S. new-haw	3,15	e,h	1,7
	S. newington	3,15	e,h	1,6
E₃	S. canoga	(3),(15),34	g,s,t	
	S. harrisonburg	(3),(15),34	z₁₀	1,6
	S. illinois	(3),(15),34	z₁₀	1,5
	S. minneapolis	(3),(15),34	z₁₀	1,6
	S. thomasville	(3),(15),34	y	1,5
E₄	S. senftenberg	1,3,19	g,s,t	
	S. simsbury	1,3,19	z	
	S. taksony	1,3,19	i	z₆
F	S. aberdeen	11	i	1,2
	S. subulasa	11	r	e,n,x
G	S. cubana	1,13,23	z₂₉	
	S. grumpensis	13,23	d	1,7
	S. poona	13,22	z	1,6
	S. telchis	1,13,23	e,h	
	S. worcester	1,13,23	z,h	e,n,x
	S. westhington	1,13,23	l,w	
H	S. florida	(1),6,14,25	d	1,7
	S. madelia	(1),6,14,25	z₁₀	
	S. onderstepoort	(1),6,14,25	e,h	1,5
	S. siegburg	6,14,18	...	
I	S. gammarus	16	d	1,7
	S. hastingfoss	16	b	e,n,x
Further groups	S. alachua	35	z₄,z₂₃	
	S. berkeley	43	e,h	
	S. cerro	...	z₄,z₂₃	
	S. champaign	...	z₄,z₂₄	1,5
	S. johannesburg	1,40	l,v	e,n,x
	S. mgulani	38	i	1,2
	S. minnesota	21	b	1,2
	S. pomona	28	y	1,7
	S. tel-aviv	28	z	e,n,z₁₅
	S. urbana	30	b	e,n,x

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Adult birds infected with paratyphoid organisms generally show no outward symptoms; however, they may serve as intestinal carriers of the infection over long periods of time. Paratyphoid infections in their exhibit little or no selectivity in their pathogenicity for specific strains or breeds of birds.

Pomeroy (1944) reported on the mortality of young poults experimentally infected with *S. typhi-murium* at 2 and 4 days of age. The mortality of the poults infected when 2 days old varied from 40 to 60 per cent, and that of the 4-day-old group was 40 per cent. Other experimental evidence indicated that the older the poults were when exposed, the lower was the expected mortality. Bierer (1960) found turkey poults extremely susceptible to *S. typhi-murium* infection during the first 48 hours after hatching. Mitrovic (1956) reported that 1-day-old turkey poults were very susceptible to experimental infection with *S. reading* with a mortality of 40 per cent, while 2-week-old poults possessed extremely high resistance to the infection. Yamamoto et al. (1961a) studied the shedding of Salmonella organisms in the feces of orally infected adult turkeys. They found that there was a marked decrease in the number of organisms shed by 14–21 days.

In contrast to poults, chicks usually do not exhibit high mortalities when infected experimentally. Watanabe et al. (1959b) in studies of the resistance of chick embryos to *S. senftenberg* infection found that the lethal dose of the organism increased in direct relation to the age of the embryos. Bliznakov et al. (1963) demonstrated that resistance of embryonated eggs to *S. typhimurium* infection may be qualitatively increased by antiserum, implantation of splenic tissue from normal or immune adult fowl, or by a combination of these treatments. Milner and Shaffer (1952) conducted detailed bacteriological studies of experimental Salmonella infections in chicks and were able to demonstrate that infection by the oral route decreased rapidly with advancing age. Fatality rates in the chicks experimentally infected were not high, although bacteremia was easily demonstrated through blood culture. Clemmer et al. (1960) found a wide variation in the response of chicks exposed to aerosol infection with various Salmonella types. *S. typhi murium* was found to be very invasive for lung tissue in contrast to the other

types studied. Beattie (1960) reported that *S. thompson* had no ill effects on chicks over 3 weeks of age without any therapeutic measures. Buxton and Gordon (1947) were able to produce a 44 per cent mortality in chicks infected orally with *S. thompson*. Approximately 70 per cent of the survivors remained intestinal carriers at 21 days of age.

Mitrovic (1956) found that either 1-day- or 2-week-old chicks were highly resistant to oral infection with *S. reading*. Sieburth and Johnson (1956) reported that orally administered *S. typhi-murium* organisms are very infective for the day-old chick with 100 per cent infection arising from 10^{20} and 50 per cent mortality from $10^{3.5}$ viable organisms per chick. Sieburth (1957a) was able to produce a cumulative mortality of 27 percent in chicks 12 days post inoculation by administering approximately 160 viable *S. typhi-murium* cells orally to each bird at 1 day of age. *S. typhi-murium* recovery from the intestine and organs was 100 per cent. Bierer (1961) found that *S. typhi-murium* infection could be induced experimentally in chicks by spraying broth cultures of the organism into incubators 1 day before hatching. Mortality was almost tripled in infected groups maintained in unheated brooders for a 10-day period. Hamada et al. (1958) found that chicks infected in the incubators usually acquire the infection through the respiratory and digestive organs, and carry the greatest concentration of the organisms during the first week of life. They may appear healthy and usually eliminate the infection in 4–6 weeks without therapy.

Shaffer et al. (1957) found marked variation in the response of day-old chicks to either peroral or parenteral inoculation with various antigenic types of Salmonella. The course of infection produced by *S. typhi murium* included marked shedding of the organisms in the feces; frequent invasion and localization in tissues such as the spleen, liver, and lungs; and deaths from bacteremia. *S. paratyphi A* exhibited less evidence of invasiveness and caused no mortality. Henderson et al. (1960) admin-

istered 7 different Salmonella serotypes orally in graduated doses to inbred White Leghorn day-old chicks. All 7 serotypes produced mortality varying from about 2 per cent (*S. anatum*) to 80 per cent (*S. typhi-murium*). Exposure to their own feces of chicks infected with *S. anatum* significantly increased but did not extend the period of mortality. Akiyama (1961) found that there was a low mortality rate in chicks artificially infected with *S. senftenberg* and the infection was rapidly shed by adult chickens.

Experimental efforts to produce chronic paratyphoid infections in both chicks and poults by oral administration of the organisms often result in the production of a disease of a transitory nature. During and a few weeks following the acute phase of the infection both tissues and intestinal cultures may readily yield positive isolations of the organisms administered. However, 1 or 2 months following oral infection most birds will be culturally negative. These findings have been very adequately substantiated by the experimental studies of Pomeroy and Fenstermacher, 1939; Gibbons and Moore, 1946; Buxton and Gordon, 1947; Gauger and Greaves, 1947; Wilson, 1948; Milner and Shaffer, 1952; Adler *et al.*, 1953; and Sieburth, 1957a. In actual field cases, the course of the disease may be longer than would be expected under controlled, experimental conditions.

Mortality from paratyphoid infection under natural conditions varies depending upon the environment, strain of infecting organism, and the presence of concurrent infections. Schalm (1937) in studies of *S. typhi-murium* infection in chicks found a great contrast in the low mortality among chicks kept on the farm where hatched, in comparison to the high mortality among chicks sold to others and transported to new quarters. Sieburth and Johnson (1956) found that the bluecomb agent, when administered to susceptible chicks in conjunction with *S. typhi-murium*, increased the mortality rate from 29 to 67 per cent. Biddle and Cover (1957) reported the isolation of Salmonella organisms from the respiratory tracts of chickens, some of which were infected with chronic respiratory disease.

HOST DISTRIBUTION

Paratyphoid infections occur in most species of warm- and cold-blooded animals. The organisms seldom exhibit host specificity. The frequent occurrence of *S. typhi-murium* var. *copenhagen* in pigeons is a notable exception. Buxton (1958), in discussing host specificity of Salmonella strains, called attention to the need for fundamental information on the chemical pathology of the reactions between the bacteria and host tissues. It was felt that such information would provide a means of explaining the carrier state in Salmonella infections. Edwards *et al.* (1948a) reported 111 serological types of Salmonella which were encountered in a total of 47 warm- and cold-blooded animal species.

Among domestic poultry, paratyphoid infections are most frequently encountered in turkeys and chickens. Turkeys are especially susceptible to these infections. The incidence of paratyphoid in chickens has revealed an increase during recent years as evidenced by the reports of Burr *et al.* (1957), Angstrom (1957), and Sieburth (1957a).

Pfaff (1921) was the first to report on the occurrence of paratyphoid infection in turkeys. Rettger *et al.* (1933) described the disease in young poults in the United States. Lee *et al.* (1936) encountered an acute disease that caused 90 per cent mortality among poults less than 5 weeks of age. In other flocks the losses were less, ranging from 40 to 70 per cent. The organism recovered from the poults was found to be pathogenic for chicks, poults, guinea pigs, and rabbits.

Cherrington *et al.* (1937) found *S. typhi-murium* to be responsible for the loss of many poults on several turkey ranches in Idaho. The mortality was as high as 60 per cent during the first week of brooding. Pomeroy and Fenstermacher (1939) first observed this infection in turkeys in Minnesota in 1932 when four poults were

gated had occurred in the southern part of New Jersey as had those reported previously by Moore and Mohler.

Lahaye and Willems (1927) considered paratyphoid to be one of the most important diseases of pigeons in Belgium. Khalifa (1935) investigated an epizootic among pigeons in Cairo, Egypt, which was due to *S. typhi-murium*. Jungherr and Wilcox (1934) reported a variant of *S. typhi-murium* recovered from spontaneously infected squabs. Morcos (1935) studied an outbreak among pigeons in various lofts in Cairo. The organism isolated closely resembled *S. anatum*. Adult fowl and sparrows were found to be quite refractive to the isolated culture. Edwards (1935) reported the close association of *S. typhi-murium* var. *copenhagen* with pigeons. Hoffmann and Edwards (1937) isolated paratyphoid organisms from pigeons which were believed to have transmitted the infection to rabbits on the same premises. Shirlaw and Iyer (1937) encountered an unusual loss among pigeons that were being used in the production of fowl pox vaccine. The organism isolated was *S. enteritidis*. Niemeyer (1939) recovered an organism from pigeons that was identified as *S typhi-murium*. The organism was recovered from 3 of 14 pigeons examined.

Gauger *et al.* (1940) published a comprehensive study of pigeon paratyphoid. The etiological type was *S. typhi-murium* var. *copenhagen*. These authors listed 26 references to paratyphoid epizootics in pigeons. Edwards *et al.* (1948c) found that 97.5 per cent of all cultures of *S. typhi-murium* isolated from pigeons were *S. typhi-murium* var. *copenhagen*. Moran (1961b) reported a similar high incidence of this variety in pigeons. This organism, unlike typical *S. typhi-murium* strains, lacks the somatic antigen 5. It is a unique example of a paratyphoid type exhibiting host specificity, and has resulted in most investigators suspecting direct or indirect association with pigeons as the source of infection when this type is encountered in other species of animals. Van Dorssen (1955) reported a serological

and cultural study of 223 *S. typhi-murium* strains isolated from pigeons in the Netherlands. He concluded that there does not exist any specific pigeon type of this organism as has been reported by some workers. Pigeons surviving paratyphoid outbreaks often become chronic carriers, excreting the organisms intermittently in their feces. Epizootics may occur among adult flocks, especially if their resistance is lowered by other conditions.

Edwards *et al.* (1948c) reported 10 serological types of Salmonella isolated from pheasants. *S. bredeney* was recovered from 7 outbreaks.

Graham (1936) studied an outbreak of paratyphoid among quail in which *S. oranienburg* was found to be the causative organism. Cunningham (1941) encountered an acute paratyphoid infection among quail chicks in which the heaviest mortality occurred in chicks from 3 to 9 days old. *S. bredeney* was isolated as the causative organism.

Hinshaw *et al.* (1942) isolated *S. bredeney* and *S. typhi-murium* from a group of chukar chicks. This report clearly indicated the isolation of multiple Salmonella types from a single outbreak wherein a 48.2 per cent mortality occurred among a group of 1,061 chicks. Francis *et al.* (1960) reported the isolation of *S. derby* and *S. anatum* from chukar partridges.

Beaudette and Edwards (1926) investigated paratyphoid in canaries and parrots. In one bird store, 200 birds of all ages became infected. The mortality was 35 per cent. *S. typhi-murium* was found to be the causative organism. Emmel and Stafseth (1929) reported several outbreaks of an epizootic of paratyphoid that occurred in canary bird stores throughout the state of Michigan. The disease was highly infectious, and the mortality was high. The incubation period was 4 or 5 days and the course of the disease varied from 2 to 4 days. *S. typhi-murium* was isolated from the internal organs.

Beaudette (1926a) reported that *S. typhi-murium* readily infected parrots as well as canaries. No differentiation could

be made between the strains of *S. typhimurium* isolated from the parrots and those previously isolated from canaries. Meyer and Eddie (1934, 1939) reported the isolation of Salmonella organisms from tropical psittacine birds including parrots, parrotlets, paroquets, and conures. Altman (1940) studied an outbreak of paratyphoid among a group of 170 canaries of all ages. The incidence of infection and mortality was greater in the young birds. Approximately 60 per cent of the infected birds died. *S. cholerae suis* was isolated as the etiological agent. Keymer (1959) reported that heavy losses occur in canaries and other passerine birds from *S. typhimurium* infection.

Meyer (1912), Buxton (1957a), and Kaye *et al.* (1961) have reported on the isolation of Salmonella organisms from budgerigars (parakeets). Meyer (1912) demonstrated that 37 per cent of parakeets from a single dealer were infected with *S. typhimurium*. Burkhart *et al.* (1962) experimentally infected parakeets and canaries with *S. typhimurium* and *S. heidelberg*. Stone (1960) cited Salmonella as an infectious cause of gastroenteritis in parakeets as encountered in veterinary practice.

Buxton (1957a) reported the isolation of *S. panama*, *S. paratyphi B*, and *S. typhi* from sea birds. Brest Nielsen (1960) isolated *S. typhimurium* from seagulls in Denmark, and Williams and Dodson (1960) reported the isolation of 3 Salmonella types from gulls. Salisbury (1958) reported that from 1948 to 1957 *S. typhimurium* was isolated from a seagull, mallard duck, pigeon, parakeet, goose, and pheasant as well as other species of poultry in New Zealand. Strauss *et al.* (1957) isolated *S. typhimurium* from the black-headed gull.

Sieburth (1958a) was unable to isolate any Salmonella organisms from the livers or intestines of 17 penguins cultured during a survey in the antarctic region. A history of salmonellosis was suggested in the ringed penguin, skua gull, and 2 sheath bills by serological tests.

Hudson (1942) encountered an outbreak of paratyphoid infection among a flock of

guinea fowl with a reported loss of 60 birds during a period of 6 weeks. *S. bredeney* was isolated from the infra-orbital sinuses.

Manninger (1913) examined the intestinal flora of various birds. From three birds belonging to the family of finches, he recovered *S. paratyphi B*. Keymer (1959) reported that he has observed *S. typhimurium* infection in the gouldian finch, the black-crested finch, the cordon bleu, the cut-throat, and the domestic variety of the Bengalese finch. Gordon and Buxton (1946) reported the isolation of paratyphoid organisms from a sparrow, Deom (1960) isolated a strain of *S. california* from a sparrow for the first time in the Congo. Dôzsa (1961) reported the isolation of *S. typhimurium* from the intestinal tract of 52 out of a total of 266 captured sparrows in Budapest. Edwards *et al.* (1948c) reported the isolation of paratyphoids from the following avian species not mentioned above: a peafowl, partridges, Japanese robins, a secretary bird, a diamond dove, a yellow-winged sugar bird, and callistes. Moran (1961b) reported the isolation of *S. typhimurium* from the following types of "other birds": cockatoo, hoatzin, hornbill, and parrot.

Cope *et al.* (1955) in a study of Salmonella in animals, birds, and reptiles isolated 8 types of paratyphoid organisms from a total of 20 species of fowl maintained in the Detroit Zoological Park. Csiszár *et al.* (1961) studied salmonellosis among birds of a zoological garden and found that approximately 27 per cent of the infected birds exhibited gastroenteritis. Such infections were not deemed to constitute a hazard to zoo visitors, but the danger for attendant personnel was stressed. Bigland *et al.* (1962), following a typing survey in Alberta, Canada, extending for 12 years, expressed the opinion that Salmonella organisms are seldom found in free-living wild birds and animals.

Hudson and Tudor (1957) isolated *S. typhimurium* from several varieties of free-flying birds including starlings, sparrows, rusty blackbirds, and a cowbird originating from 4 outbreaks in 3 areas of

north central New Jersey during a period of 2 years. They called attention to the possibility that these birds may spread the infection to man and domestic animals. Vallée et al. (1959) reported the isolation of *S. johannesburg* from Bengali birds during an enzootic in a commercial bird shop. No treatment was found effective. Petzelt and Steiniger (1961) isolated 18 types of Salmonella from chaffinches, house sparrows, black-headed gulls, starlings, and blackbirds captured in the area of a sewage purification plant of a big city.

The reader seeking additional historical information on paratyphoid outbreaks in various species of fowl is referred to the comprehensive review of Henning (1939).

Unlike the causative organisms of pullorum disease and fowl typhoid, the paratyphoids are common pathogens of most species of domestic and wild mammals and man. Cattle, swine, sheep, goats, dogs, cats, horses, mink, and foxes are among the many animal species that may be chronically infected and shed the organisms in large numbers in their feces. In these animals, paratyphoid usually occurs as an acute disease only in the very young or in old, debilitated animals under extreme conditions of stress. A voluminous amount of literature has been accumulated on Salmonella infections of animals other than poultry and for a review of this subject the reader is referred to Buxton (1957a).

Craige (1944), Wolff et al. (1948), Adler et al. (1951), Galton et al. (1952), Stucker et al. (1952), McElrath et al. (1952), and Mackel et al. (1952), have reported studies of paratyphoid isolations from the feces of dogs. Dogs and cats often carry Salmonella organisms in their digestive tract without showing any clinical symptoms. Bruner and Moran (1949) reported 26 Salmonella types recovered from dogs. Approximately 40 per cent of the cultures were *S. typhi-murium.* Thirty-four cultures isolated from cats included 17 types of Salmonella. Jungerman and Grumbles (1960) isolated Salmonella organisms from 9 of 100 mature healthy dogs

studied. Two of the dogs were infected with more than one Salmonella type. The infection was of a transient nature and cultures were negative 6 weeks later.

Rats and mice are frequently intestinal carriers of paratyphoid organisms, particularly *S. typhi-murium* and *S. enteritidis.* When *S. enteritidis* is encountered in poultry it is logical to suspect these rodents as a possible source of the infection. Salmonella have also been isolated from various insects including flies, fleas, and cockroaches. It is known that *S. enteritidis* can be transmitted through the complete life cycle of flies and that the infection may continue as long as 4 weeks within flies (Ostrolenk and Welch, 1942; Greenberg, 1959). Kaye et al. (1961) noted paratyphoid isolations from the housefly, tick, louse, flea, and cockroach. Trawiński and Trawińska (1960) were able to isolate Salmonella from artificially infected houseflies, their larvae and pupae. Buxton (1957a) cited references indicating that ticks may remain carriers for more than 30 days after oral infection.

McNeil and Hinshaw (1946) isolated *S. san-diego* and *S. newport* from Galapagos turtles, *S montevideo* from a Gila monster, and *S. manhattan* from an iguana. Hinshaw and McNeil (1945) examined 41 snakes caught on ranches in 7 localities in California. Eleven of the snakes yielded Salmonella on culture. Bövre and Sandbu (1959) isolated 19 types of Salmonella from 27 tortoises in Oslo.

Most of the Salmonella types recovered from poultry have also been found to infect man causing gastroenteritis or occasionally a more serious septicemic type infection. The role of eggs and poultry products in the transmission of Salmonella infections to man has been reviewed by Galton (1956), McCullough (1958), and Galton and Arnstein (1960).

Hinshaw et al. (1944) recorded the transmission of 2 types of paratyphoids (*S panama* and *S. montevideo*) to man believed to have occurred as a result of handling infected poults. Kaye et al. (1961) described a case of *S. typhi-murium* infec-

tion in an infant who was allowed to crawl in the droppings of a pet parakeet that was infected. Hinshaw and McNeil (1918, 1951) recorded 7 cases of gastroenteritis among poultry caretakers resulting from contact with acute outbreaks of pacatyphoid in fowl. Darby and Stafseth (1942) reviewed the literature with reference to 35 types of the genus *Salmonella* found in poultry in the United States. Most of these types have also been incriminated in pathological conditions in man. Cherry et al. (1946) isolated a nonmotile Salmonella from frozen turkeys. Browne (1919) was able to isolate Salmonella from the skin of turkeys that had been frozen for 13 months. Schneider and Gunderson (1919) recovered 4 Salmonella types from the skin of 4.4 per cent of 1,014 eviscerated chickens. Felsenfeld (1919) reported a case of human infection with *S. cubana* from eating a New York dressed chicken infected with this organism.

Felsenfeld et al. (1950) in a survey of Salmonella organisms in market meat, eggs, and milk called attention to the need for proper inspection of certain foods intended for human consumption. Brandly (1951) and Dolman (1954) have also emphasized the public health significance of Salmonella infections of poultry. Abelseth and Robertson (1953) implicated *S. typhimurium* infection of turkeys as a definite public health hazard. Anderson et al. (1955) reported several cases of *S. typhimurium* infection in children with a likely source of the infection being Easter chicks. *S. typhi-murium* was recovered from the stools of the children and also the chicks.

Gunderson et al. (1954) and Galton et al. (1955) have discussed the occurrence and distribution of paratyphoid organisms in poultry processing plants. Florin and Nilsson (1959) cited the very important influence of techniques in slaughter on the contamination of carcasses in the case of intestinal salmonellosis of poultry. Recognizing this fact, many poultry processing plants in the United States are establishing laboratory control testing services to monitor the presence of Salmonella organisms in their operations. If results warrant, necessary measures are taken to improve sanitary conditions within the plant.

Savage (1956) noted that the incidence of Salmonella food poisoning in Great Britain was increasing. Meat and egg products were cited as the chief sources of the infection in man. Canale-Parola and Ordal (1957) found 5 out of 40 poultry pies sampled to contain Salmonella organisms. They recommended that manufacturers pled technology and sanitary procedures used in preparing the pies and give longer baking periods. Similar studies have been reported by Litsky et al. (1957). Beloian and Schlosser (1963) reported that baked foods that reach a temperature of 71° C. (160° F.) or higher in the slowest heating region can be considered safe from any Salmonella organisms that may be present in the ingredients.

Edwards (1958) in an excellent review emphasized the importance of poultry, and animal species in general, in the transmission of salmonellosis to man. Brobst et al. (1958) were successful in isolating Salmonella from chickens and ducks being prepared for market as well as from vats in poultry processing plants in Western Pennsylvania. They concluded that potentially these Salmonella are a serious menace to those who handle the birds and to the families that may receive them. Van Keulen (1959) described the role played by animal-borne salmonellosis in human infections. Attention was drawn to the significance of infection through the medium of foodstuffs of poultry and animal origin, whether primarily or secondarily infected. Better hygiene in the production and handling of foodstuffs of animal origin was stressed. Mackel et al. (1959) studied an outbreak of human gastroenteritis due to *S. typhi-murium* among 300 inmates of a penal institution. The patients had eaten roasted turkey which had been sliced on the same chopping block on which the uncooked fowl had been prepared. Those persons eating only reheated meat did not become ill. *S. typhi-murium* was recovered from

more than 100 persons that had eaten the meat as well as from turkey necks that had been frozen. Spink (1960) traced an outbreak of *S. thompson* infection involving 35 people in Great Britain to a broiler shop. The organism was isolated from cooked chicken, the manager of the shop, his assistant, and from living broilers at a packing station supplying the shop.

Walker (1960) called attention to the increased incidence of salmonellosis in humans in Great Britain, and cautioned that the use of antibiotics in broiler rations is resulting in the emergence of resistant Salmonella strains. Such strains may cause an increase in the carrier rate and may multiply more rapidly in contaminated carcasses. It has been suggested (Anon., 1961) that the carcasses of poultry for the market may be immersed in slush ice containing 10 p.p.m. of chlortetracycline for 2 hours to inhibit the growth of Salmonella. Attention is called to the development of chlortetracycline-resistant strains of Salmonella as this drug may be given to the birds as a feed additive. Garside *et al.* (1960) reported that birds receiving antibiotics for the prevention or treatment of Salmonella infections may be a public health hazard because of the large number of infected carrier birds remaining in such flocks.

Morris and Ayres (1960) found that 0 to 9 per cent of the samples obtained from turkey processing plants and 7 to 14 per cent of the samples from chicken processing plants were positive on culture for Salmonella. Specimens were taken from 10 sampling stations including the scald tanks, carcass surfaces, and final rinse water. Twenty-eight Salmonella cultures were isolated from chickens and 12 from turkeys. Tailyour and Avery (1960) cultured the viscera and intestinal contents of 525 market weight turkeys in Vancouver, British Columbia, for Salmonella. Four birds (0.76 per cent) were found to be harboring *S. derby*. Wilson *et al.* (1961) found approximately 17 per cent of 525 raw poultry specimens in the Cincinnati area contaminated with Salmonella. Eighteen serotypes were encountered during the study

and Salmonella isolations showed an increasing trend from supermarket through general store, meat market, and poultry market. Eight per cent of household turkey specimens were positive for Salmonella.

Edwards (1958) and Quist (1962) reported a 7-fold increase of Salmonella infections other than typhoid fever in humans in the United States from 1949-1960 and noted that Salmonella infections in poultry constitute the major source of human disease. Sadler *et al.* (1961) random-sampled market meat birds for Salmonella organisms. Infected or carrier birds were found being processed on 43 per cent of the sampling days for turkeys, 26 per cent for chicken fryers, and 12 per cent for hens. Dixon and Pooley (1961) isolated Salmonella organisms from 15.8 per cent of 544 specimens taken at a poultry plant in Great Britain.

Raw, frozen, or dried eggs are rather frequently implicated in Salmonella infections in man. An outbreak due to raw eggs containing *S. tennessee* was reported by Watt (1945). Blaxland and Blowers (1951) implicated *S. typhi-murium* in duck eggs as a cause of human food infection. Such occurrences have been frequently reported in Europe.

The occurrence of Salmonella in egg powder has been extensively studied by Schneider (1946) and Solowey *et al.* (1946, 1947). McCullough and Eisele (1951a, b, c, d), in detailed clinical studies of experimental salmonellosis in human volunteers, were able to establish the pathogenicity of 6 Salmonella types which had been isolated from spray-dried whole egg powder. Cultures were administered in graduated doses in eggnogs and the following Salmonella types were used in these studies: *S. meleagridis, S. anatum, S. newport, S. derby, S. bareilly,* and *S. pullorum.*

Alves de Oliveira and Gomes (1954) described a hospital epidemic of *S. typhi-murium* infection involving 200 persons and resulting in 3 deaths. The outbreak was traced to eggs consumed in the diet. Newell *et al.* (1955) traced 2 outbreaks of

paratyphoid fever (*S. paratyphi B*) in humans to the consumption of cream-filled cakes. The same phage type of *S. paratyphi B* was found in unopened cans of Chinese frozen whole egg used by the bakeries, in some members of the staff, and in the patients. An anonymous report (1956) disclosed that in 1955 in Great Britain, there was an increase of 49 per cent in food poisoning, due principally to Salmonella infections frequently traced to egg products. Buxton (1957b) called attention to the fact that human foods composed of eggs and egg products are a common source of Salmonella food infection in man. It was emphasized that the rate of infection is much higher among eggs with cracked and fecal-contaminated shells than among those laid under clean, hygienic conditions.

McCullough (1958) discussed the importance of poultry and poultry products, especially eggs, in the epidemiology of salmonellosis in humans in the United States. In one epidemiological investigation, *S. infantis* was isolated from poultry feedstuffs, eggs, and patients that had eaten lightly cooked and uncooked egg products (Newell *et al.*, 1959). Taylor (1960) called attention to the importance of eggs and egg products in the transmission of Salmonella infections in man. It was noted that the infection is more often traced to frozen eggs, dried eggs, and other egg products.

Philbrook *et al.* (1960) reported an outbreak of *S. typhi-murium* food poisoning traced to infected chicken eggs consumed in eggnogs by patients at an institution in Massachusetts. Eggnogs consumed in the institution are now pasteurized. Fey and Wiesmann (1960) described a family outbreak of salmonellosis in Switzerland caused by imported egg powder which contained 5 different Salmonella types. Attention has been called (Anon., 1961) to the public health significance of Salmonella in poultry and the fact that eggs that may be contaminated with Salmonella should be cooked well. Hobbs (1961) referred to the importance of poultry products in transmitting salmonellosis to humans, and listed pasteurization of egg products and

the pelleting of animal feeds as factors that would help to reduce this transmission. Thatcher and Montford (1962) reported that 14 different Salmonella types were isolated from 119 egg-containing cake mixes of which 65 contained Salmonella. The importance of breaking the animal-to-animal chain of infection in the control of salmonellosis was emphasized. Taylor (1963) has reviewed the Salmonella serotypes isolated from egg products and found in human infections in the United Kingdom. During a hospital-associated epidemic of *S. derby* infection (Anon., 1963a) in the United States it was recommended that cracked and unclean eggs should not be used. It was further suggested that commercial products containing eggs or egg products should be cooked well.

MODES OF TRANSMISSION

The wide distribution of paratyphoid organisms under natural conditions contributes materially to their rapid spread. Preventive efforts to be successful must give first consideration to the means by which the disease is transmitted. The frequent isolation of paratyphoid organisms from eggs has resulted in the reference to the disease as an egg-borne infection. However, in consideration of the spread of paratyphoid infections through the medium of the egg, it is important that a distinction be drawn between direct ovarian transmission and transmission through shell penetration.

Numerous investigators (Dalling and Warrack, 1932; McGaughey, 1932; Warrack and Dalling, 1933; Wilson, 1945; Karlshøj and Szabo, 1949) have recovered paratyphoid organisms from the yolk of duck eggs, providing ample evidence that direct ovarian transmission is quite common in this species. Hole (1932) and Clarenburg (1939) have also presented definite evidence that duck eggs may be infected as a result of localization of the organism in the ovary.

Paratyphoid infections of turkeys may occasionally be directly transmitted through the ovaries. However, experimen-

tal evidence does not indicate that infected turkeys produce a high percentage of infected eggs. Cherrington et al. (1937) reported the isolation of S. typhi-murium from the ovaries of two turkeys. The results of these investigations indicated that the infection was transmitted from the ovaries of the breeding hens to fertile eggs. Lee et al. (1936) investigated the direct ovarian transmission of S. typhi-murium infection of turkeys and were able to recover the organism from the ovarian tissues of infected hens. Pomeroy and Fenstermacher (1939) reported the isolation of S. typhi-murium from the ovary and oviduct of 3 of 10 naturally infected turkeys. Hinshaw and McNeil (1913a) were able to isolate S. typhi-murium from the ovaries and oviducts of turkeys. Gauger and Greaves (1946a) isolated S. typhi-murium from the ovary of a turkey hen. Due to the very short incubation period that was encountered in a natural outbreak of S. reading infection in poults, Mitrovic (1956) suggested the likelihood of direct egg transmission. Yamamoto et al. (1961a) reported the isolation of S. typhi-murium from the ovaries of an adult turkey that was experimentally infected.

There is little evidence in the literature to suggest that direct ovarian transmission is a common occurrence in chickens. Wilson (1950) reported the isolation of S. typhi murium from chicken eggs, and suggested the possibility of direct ovarian transmission. Clarenburg and Romijn (1954) were successful on one occasion in isolating S. bareilly from the ovary of an infected chicken. Others have made similar reports; however, isolations of paratyphoid organisms from ovarian tissues of chickens are not common. Wilson (1948) stated that "it seems certain that direct egg transmission of infection rarely, if ever, occurs in chicks as most workers have consistently failed to isolate the organism in the content of chicken eggs." This is in direct contrast to the cycle of pullorum disease in chickens. Mundt and Tugwell (1958) were unable to recover Salmonella organisms from the contents of eggs laid by White Leghorn pullets experimentally infected orally and intravenously with various types of paratyphoid organisms. However, the organisms were recovered from shells of eggs laid by the most severely infected group 21 days after infection and from feces 35 days after infection. Snyser and Van Roekel (1959) cultured eggs of reacting chickens from a flock known to be infected with S. typhi-murium. The organism was isolated from the eggs on one occasion.

Fecal contamination of eggshells with paratyphoid organisms during the process of laying or from contaminated nests or incubators after laying is of foremost importance in the spread of the disease. Intestinal carriers of the organisms are common (Emmel, 1936a; Mallmann et al., 1942; Posell, 1942; Hinshaw and McNeil, 1913a; Gauger and Greaves, 1916c, 1917; Gibbons and Moore, 1916; Chase, 1917; Milner and Shaffer, 1952; Adler et al., 1953; Sieburth, 1957a). Buxton and Gordon (1917) demonstrated that chickens may remain intestinal carriers of S. thompson up to 14 months. Yamamoto et al. (1961a) inoculated S. typhi-murium into the crop of adult turkeys and found that the organisms were generally shed in higher numbers in the cecal than in the other intestinal feces. Cloacal swabs were positive (83.4 per cent) at 14 days and also (27.8 per cent) at autopsy 35 to 44 days postinoculation. Total positive isolations were increased to 67 per cent when all methods of culture (swab, direct fecal, and visceral organs) were considered.

Perek and Rabinovitz (1957) were able to obtain positive fecal cultures of S. enteritidis for a period of 3 months from a naturally infected adult flock of geese. After 7 months, fecal cultures were negative. Bacteriological examination of all internal organs was also negative at that time. Pulst (1960) found that geese infected orally with S. typhi-murium excreted the organism for 18–26 days after infection, and one goose became a chronic carrier. All of the birds developed agglutinin titers which disappeared in about 4 weeks.

Schalm (1937) was able to demonstrate that *S. typhi-murium* in fecal material smeared on the surface of chicken eggs was capable of penetrating the shell and multiplying within the egg. These findings suggested a means by which paratyphoid organisms might be introduced into the incubator by contaminated eggs with subsequent spread to the hatched chicks or poults. Maclaury and Moran (1959) found that freshly laid eggs, cooling in contact with contaminating organisms, will draw the organisms through the shell pores. The bacteria may be recovered on the day of contamination from the inside of the shells of 84 to 95 per cent of the eggs. Beach (1936) also called attention to the role of contamination on the eggshell in the transmission of the infection. Buxton and Gordon (1947) noted that the average diameter of the pores of chicken eggs varied from 6 to 13 micra, which would readily permit paratyphoid organisms to penetrate the shell when conditions are favorable.

Wright and Frank (1956) demonstrated that a few eggs are penetrated by *S. typhi-murium* on the first day and the great majority by the end of the seventh day. They also found that the specific gravity of the eggs was related to shell thickness, and there was an increase in the proportion of eggs penetrated in the low specific gravity groups. Beattie (1960) found that embryonic infection with *S. thompson* was highest during those months of the year when environmental temperatures are lowest, indicating a rapid shell penetration.

Pomeroy and Fenstermacher (1939) incubated 325 eggs from turkeys that had shown positive agglutination reactions to one or more paratyphoid antigens. Ninety of the eggs were infertile, 103 contained dead embryos, 7 contained poults which had pipped the shells but failed to hatch, and 115 hatched. On bacteriological examination *S. typhi-murium* was recovered from 3 infertile eggs (3.33 per cent), from 8 eggs containing dead embryos, and from 1 egg containing a dead poult. In further studies Pomeroy and Fenstermacher (1941) found that *S. typhi-murium* organisms,

when mixed with sterile turkey feces and smeared on one-third of the surface of turkey eggs, were able to penetrate the eggshell during incubation and invade the egg contents. In one group 17.6 per cent, and in a second group 16.0 per cent of the eggshells were penetrated.

Gauger and Greaves (1946a) examined the contents of eggs from turkeys naturally and artificially infected with *S. typhi-murium*. The organism was not recovered from the contents of 164 eggs laid by 6 naturally infected carrier hens although these birds had voided *S. typhi-murium* in their feces quite regularly during the period of the experimental study. Thirty-four of the eggs cultured were laid by one bird found to have *S. typhi-murium* ovarian infection. *S. typhi-murium* was recovered from the shell of 27 eggs and shell and contents of 6 of 117 eggs laid by a group of turkeys experimentally infected with *S. typhi-murium*. These studies provided additional evidence of the importance of shell contamination in the transmission cycle of paratyphoid infections. Yamamoto *et al.* (1961a) found that *S. typhi-murium* could be recovered from the surface of eggs laid by experimentally infected adult turkeys; however, subsequent culture of the contents of incubated eggs yielded negative results.

Wilson (1945) isolated *S. typhi-murium* and *S. thompson* from the outside of eggshells and suggested that the mixing of such eggs with clean eggs is a means of spreading the infection in the incubator. Buxton and Gordon (1947) demonstrated that *S. thompson* could readily penetrate the shell of chicken eggs stored at 37° C., but penetration was less common in eggs stored at room temperature. Gregory (1948) reported the penetration of *S. typhi-murium* and *S. bredeney* through the shell of chicken and turkey eggs under different conditions of humidity.

Cantor and McFarlane (1948) isolated strains of *S. montevideo* and *S. anatum* from 13 (0.6 per cent) of 2,132 samples of eggshell scrapings. Wilson (1948) reported the examination of 1,023 chicken

eggs, from 60 of which S. thompson was isolated. Forty-five of the eggs yielded the organism from the outside of the shell and 17 from the contents. It was felt that penetration of the shell readily occurred as the eggs were stored for a period of 14 days and no effort was made to keep the various eggs apart. Watanabe et al. (1958) were unable to isolate S. senftenberg from the eggs of adult hens that were artificially infected with the organism and reacted positively to both somatic and flagellar antigens of S. senftenberg. These workers were able, however, to isolate S. senftenberg from the shell membranes of dead embryonated eggs, suggesting that these organisms may invade the shell during incubation. Clarenburg and Romijn (1951) cultured 160 eggs which were placed in an incubator after having been contaminated with a mixture of chicken feces and broth cultures of S. bareilly. Eggs were cultured daily, but in no case could Salmonella be recovered from either the eggs or the embryos. Stokes et al. (1956) demonstrated that paratyphoid organisms are able to penetrate the eggshell membrane and contaminate the yolk if a temperature favorable to the growth of the bacteria is maintained.

Temperature and moisture play a very important part in the rate of penetration of the eggshell by paratyphoids. Schaaf (1936) found that in the case of duck eggs penetration occurred in 5 days while Lerche (1956) reported that at room temperature penetration required 15 days. Gaugusch (1958) artificially infected the surface of duck eggs with Salmonella and found that the organisms had penetrated to the interior of the eggs within 16–24 days following the infection. Formaldehyde fumigation was found to be very effective in the disinfection of infected eggs. Wilson (1948) demonstrated that the virulence of the organism is important in the rate of eggshell penetration. He found that after repeated passage, penetration of the shell could occur in 3 days. Bigland and Papas (1953) found shell penetration in 8 per cent of eggs contaminated

with S. typhi-murium, 3 per cent contaminated with S. oranienburg, and 16 per cent with S. kentucky. No penetration was found when S. bareilly was used. Ellemann (1959) found that S. typhi-murium did not penetrate the shells of eggs stored for more than 2 months at 4° C. However, penetration was demonstrated as early as 4 days when eggs were stored at 30° C. It was further noted that storage of eggs is most favorable in a cold room with a low relative humidity.

Experimental evidence indicates that penetration of the shell occurs during the first week of incubation, and to a much lesser degree in eggs stored in a cool environment. This is an important consideration in the prevention of paratyphoid infections, and will be discussed in more detail under the section on Prevention and Control.

Organisms that have gained entrance into the egg are able to multiply rapidly in the yolk and subsequently infect the developing embryo which may die or hatch to serve as a source of the infection for other young birds. Egg albumen has very little inhibitory effect on Salmonella organisms that penetrate the shell (Burton and Gordon, 1917; Gregory, 1948; Lancaster and Crabb, 1953a).

Contaminated eggshells and other debris of the hatch may also serve as a source of the infection in the incubator. From the incubator the organisms may be distributed by air currents throughout the hatchery and a high level of Salmonella aerosol infection established. Samples of air taken within the hatchery may remain positive for several weeks or months, and the infection spreads to subsequent hatches through this means. Contaminated down and dust may carry the organisms and be inhaled by susceptible young birds. Adler et al. (1953) demonstrated that poults could be infected with S. typhi-murium by the intranasal route.

Paratyphoid infection, when established in the brooder, is rapidly transmitted by inhalation, fecal contamination of feed and water, or direct consumption of fecal

ples of complete poultry feeds examined, 8 per cent yielded Salmonella. It appeared probable that the organisms were present in relatively small numbers in contaminated material, although it was stressed that multiplication may occur in mashes under suitable conditions of moisture and temperature. Graham-Jones and Fiennes (1959) isolated a Salmonella from 2 African-Grey parrots which excreted abnormally green feces. The authors were able to isolate the same Salmonella type from egg yolk material of Chinese origin included in the parrots' seed. They questioned the desirability of including such egg products in the seed of any grain-eating birds. Moran (1960) called attention to the fact that in 1957 only 60 Salmonella cultures from feed products of animal origin were typed; however, in 1958, 555 cultures of the same origin were received for typing. This sharp increase was interpreted to reflect the interest in the occurrence of Salmonella in feed products. She indicated that control of salmonellosis can begin only after strict standards of sanitation, rigidly enforced, are applied to production of animal feed ingredients.

Bischoff (1960) reviewed the world-wide distribution of 113 Salmonella types isolated from egg products and animal feeds. Strict bacteriological screening of all imported products for Salmonella organisms was emphasized. Hofmann et al. (1960) cultured duck feathers imported into Austria. Fifteen Salmonella serotypes were isolated from feathers imported from Asia; however, none was isolated from those imported from European countries or the United States. Also, the dust produced during feather cleaning contained Salmonella, but none could be isolated from the finished goods leaving the factory. As the dust is often used without heating in fertilizers, the danger of infection through this route was stressed. Schwerin (1960) reported the isolation of S. newington from gulls' droppings near a fish meal factory in which samples of fish meal occasionally yielded S newington in spite of strict hygienic measures. Taylor (1960) noted that it is both possible and practical to treat animal feed and feed constituents with heat to destroy Salmonella which they may contain. Such a practice was cited as having a potentially profound effect on the general incidence of Salmonella infections. Pelleting of poultry feeds has been suggested as an effective measure to destroy Salmonella organisms that may be present (Hobbs, 1961).

Quesada et al. (1960) found that fish meal imported into Italy from Angola was contaminated with S. binza, which was found to be pathogenic for chickens. Bischoff (1961) reported on the Salmonella types isolated from egg products and feeds of animal origin. A total of 115 Salmonella types were recovered, and the strict bacteriological control of imported products was emphasized. Giovaneli and Dominguez (1960) reported that hemp seed fed to flute canaries was the source of a natural outbreak of S. typhi-murium infection. The organism was isolated from cardiac blood and fecal material of the infected birds.

Morehouse and Wedman (1961) reported a survey of Salmonella and other disease-producing organisms in animal by-products. Egg products, poultry by-products, and meat scraps were among the feed constituents found to contain Salmonella. It was concluded that Salmonella organisms present in animal by-products and finished rations are a potential disease threat to poultry as well as other animal species. Wedman (1961) called attention to the potential disease problem resulting from the "cycling" of a number of Salmonella serotypes from farms to processing plants and back to farms by animal by-products incorporated into feeds. It was felt that realistic, workable measures to minimize the chance for exposure of poultry to Salmonella occurring in animal by-products and rations are desirable and essential. Niven (1961) pointed out some of the problems that industry encounters in efforts to eliminate Salmonella organisms from rendered animal by-products used in the manufac-

ture of animal feeds. The need for further information concerning the real significance of this problem to animal health was emphasized.

Pomcroy and Grady (1961) conducted bacteriological examinations of 980 samples of animal by-products used in manufacturing poultry feeds in 22 states and Canada. Salmonella was isolated from 175 of the samples and 43 serotypes were identified. Many of the same types have been isolated from poultry submitted to diagnostic laboratories. The authors pointed out that if continued progress is to be made in reducing the incidence of paratyphoid infections in poultry, every effort must be made to eliminate the contamination of feed ingredients with Salmonella. Thomas (1961) discussed the beneficial effects of legal measures that have been taken to restrict the use of Salmonella-contaminated ingredients in the production of animal feeds in Belgium. Hammer (1961) indicated that Salmonella infections of poultry can be traced back to the feed they receive and recommended that legal measures should be taken to minimize infection among all domestic animals through this route.

Grumbles and Flowers (1961) cultured for Salmonella 136 samples of cottonseed and/or soybean oil meal used in the preparation of poultry feeds. Six Salmonella types were isolated from 5.14 per cent of the samples. This study is especially significant since it demonstrates that Salmonella may also be present in feed constituents of vegetable origin. These authors stated that "a method of processing and handling poultry feed and feed ingredients to assure freedom from Salmonella contamination must be developed before a satisfactory control program for paratyphoid infections in turkeys can be carried out." Quist (1962) reported the isolation of 13 serotypes of Salmonella from 60 samples of protein supplement used in poultry feed manufacture. He also pointed out that two significant factors that have influenced the high rates of paratyphoid infec-

tion in poultry are infection through egg transmission and exposure of birds to feeds heavily contaminated with Salmonella organisms.

Boyer et al. (1962) described in detail two cases of salmonellosis in turkey poults and one case in chicks in which it was possible to correlate Salmonella serotypes causing the infections with those isolated from samples of feed that the birds were receiving. In two other cases the types of Salmonella isolated from the birds were not identified with the types recovered from the feed. It was emphasized that no control program for Salmonella infections in poultry will be of much value until a way can be found to keep these organisms out of the feed. Burr and Helmboldt (1962), during a one-year period, cultured 131 fish meal, 161 meat scrap, and 145 poultry by-products before incorporation into finished poultry feeds. Fifty-six Salmonella isolations were made from an average of 12.8 per cent of the samples, and 10 Salmonella types were represented. These workers called attention to cross reactions that can occur from such infections during pullorum-typhoid testing. Galbraith et al. (1962), in a survey of Salmonella organisms in pet foods and garden fertilizers in Great Britain, found 27 per cent of raw horse meat samples, 16 per cent of other raw meat, 12 per cent of prepared pet food, and 13 per cent of garden fertilizers to contain Salmonella organisms.

As Edwards (1958) has noted, it is obvious that in any effort to eradicate salmonellosis from domestic animals it is necessary to take into consideration the continuous seeding of the population through infected feedstuffs.

Rats and mice are frequent carriers of the organisms, and their droppings may readily contaminate feed supplies. Pigeons, sparrows, and various other species of wild birds may also serve as a source of the infection for domestic poultry flocks. Brest Nielsen (1960) has suggested that wild birds are a source of Salmonella infections in Danish poultry flocks. Gaugusch

and Malwińska (1956) recovered *S. typhi-murium* from natural water reservoirs on which various types of water birds lived and fed. They found such sampling of water to be an important supplementary method of diagnosis of salmonellosis in free-flying water birds.

Attention has already been drawn to dogs, cats, swine, cattle, sheep, and goats as sources of paratyphoid infections for poultry. Gwatkin and Mitchell (1911) and McNeil and Hinshaw (1911) have discussed the part that snakes, flies (*Musca domestica*), and cats may play in the transmission of the infections to poultry. Man must also be recognized as a possible source of the disease (Gordon and Buxton, 1916). This source may involve not only the caretakers but also waste products of human source. Hinshaw *et al.* (1944) record instances of humans transmitting the infection to poults. Poultry may serve as a source of salmonellosis for other animal species on the farm. Ladehoff (1959) cited evidence that *S. typhi-murium* infection in calves was introduced by apparently healthy geese and ducks.

Mortelmans *et al.* (1958) isolated 5 serotypes of Salmonella from chicks shipped into the Belgian Congo from other parts of Africa and Europe by air. They called attention to the importance of air shipments as a means of rapid spread of salmonellosis among poultry. Rao and Gupta (1961) also reported on the isolation of Salmonella from imported chickens in India.

In transmission among adult poultry the feces of infected carriers is probably the most common source of the organisms. The bacterial population and the nature of the environment are among the many factors that determine the extent of such transmission. Buxton and Gordon (1917) reported an instance in which *S. thompson* infected the gallbladder of a chicken, and the organisms were excreted in the feces for at least 18 months.

adults closely simulate those observed in pullorum disease, fowl typhoid, Arizona infections, and several other diseases. A differential diagnosis based on symptoms and necropsy findings is not possible.

Young birds. Basically paratyphoid infection is a disease of young fowl with environmental conditions, degree of exposure, and the presence of concurrent infections having an important influence on the severity of an outbreak. In acute outbreaks with deaths occurring in the incubator or during the first few days after hatching no symptoms may be noted. In such instances the infection is acquired by egg transmission or early incubator exposure. A high proportion of pipped and unpipped eggs containing dead embryos may be observed.

In experimental exposure studies of turkey poults orally administered broth cultures of *S. typhi-murium*, Pomeroy (1911) observed that losses started 2 to 3 days after exposure and discontinued by the time the poults were 2 weeks old. When symptoms are observed in young birds 1 week of age or older, contact exposure or an outside source of the disease should be considered.

Symptoms of paratyphoid infections in all species of young fowl are very similar and include the following signs: a progressive state of somnolence evident by a tendency to stand in one position with the head lowered, the eyes closed, the wings drooping, and the feathers ruffled; a marked anorexia and increased water consumption; a profuse, watery diarrhea with pasting of the vent and a tendency of the birds to huddle together near the source of heat. Bierer and Vickers (1960b) found that nitrofuran therapy of *S. typhi-murium* infection in poults reduced the incidence of soiled and pasted vents. Respiratory symptoms are not commonly observed. Clemmer *et al.* (1960) found that experimentally produced pulmonary infections in chicks were usually self-limited although 2–3 weeks may be required for autosterili-

condition in chicks which he was able to produce by parenteral inoculation of the endotoxin of *S. typhi-murium*. Rasmussen (1962) described the frequency with which *S. typhi-murium* is associated with arthritis in ducks. Lee *et al.* (1956) reported that chicks orally infected with *S. typhi-murium* died between the fifth and twelfth day, most losses occurring between the fifth and eighth day. Evans *et al.* (1955) have described blindness in chicks associated with salmonellosis. Lannek *et al.* (1962) reported conjunctivitis in natural *S. typhi-murium* infection in chicks. Salisbury (1958) found navel infection in young chicks from which *S. typhi-murium* was isolated. Bierer (1961) demonstrated that a navel route of infection was established by the incubator-spray inoculation of baby chicks with *S. typhi-murium*. The organism was also recovered from livers, unabsorbed yolks, and ceca of chicks so infected.

Dougherty (1953) and Price *et al.* (1962) found that ducklings infected with paratyphoid usually die slowly, tremble and gasp for air, and very often have pasted vents. The eyelids of ducklings frequently become swollen and edematous. Truscott (1956) reported that infected ducklings appeared drowsy and weak. Peick and Rabinovitz (1957) found that goslings with *Salmonella* infection were sluggish and refused feed or ate very little, but consumed large quantities of water. Diarrhea was observed in some birds.

Arthritis is commonly observed in paratyphoid infection of pigeons. It most frequently involves the wing joints and is evident as soft, subcutaneous swellings. Swelling of the eyelids is also a common symptom seen in pigeons. Das *et al.* (1959) reported that affected pigeons demonstrated anorexia, greenish diarrhea, drowsiness, and death within 2–3 days. Altman (1940) observed that infected canaries seemed to "puff up," developed convulsions, and died within 2 to 3 days. Consupation was observed in some cases; later, diarrhea developed. The droppings were greenish in color and, in some instances, bloody. Keymer (1959), in discussing paratyphoid infection in canaries and other passerine birds, noted that incubation takes 4 to 5 days, and the infection is usually acute. The birds will die 2 to 4 days after the plumage becomes ruffled, and greenish diarrhea is observed. Vent feathers usually become matted with droppings and convulsions often precede death. Surviving birds are apparently carriers and can infect susceptible birds.

Lesions in young birds may be entirely absent in extremely severe outbreaks. In outbreaks which permit the development of advanced cases, the following lesions are most commonly observed: emaciation, dehydration, coagulated yolks, congested liver and spleen with hemorrhagic streaks or pin-point, necrotic foci, congested kidneys, and pericarditis with adhesions. However, both heart and lung lesions are not as frequently observed as in pullorum disease. Hemorrhagic enteritis involving the duodenum is a common occurrence in poults. Cecal cores are occasionally observed. Ballantyne (1953) reported that in cases which he studied, caseous ceca were found in 33 per cent of poults infected with *S. oranienburg*, in 46 per cent of poults infected with *S. typhi-murium*, and in 20 per cent of poults with *S. newport*. The same condition occurred in 33 per cent of chicks with *S. typhi-murium* infection.

Lukas and Bradford (1954) in an effort to classify necropsy findings of paratyphoid outbreaks encountered among poults during a survey in California found: (1) systemic involvement with lesions such as pericarditis, necrotic foci in the liver and heart, air-sac involvement, central nervous system disturbance, and severe catarrhal enteritis with cecal cores (20.8 per cent of specimens); (2) uncomplicated catarrhal enteritis (33.2 per cent of specimens); (3) enteritis complicated with Coccidium, Hexamita, or sinusitis-air-sac infection (28.4 per cent of specimens); (4) other findings such as water starvation, ascites, mycosis, etc. (15 per cent of specimens); (5) no gross lesions (2.5 per cent of specimens). *S. typhi-murium* was most frequent-

ly involved in systemic cases, while other common types such as *S. anatum* and *S. manhattan* were more common in cases with uncomplicated diarrhea.

Truscott (1956), on necropsy of ducklings infected with *S. moscow*, found the livers of the birds bronze in color and covered with small grey areas of focal necrosis. The air sacs were somewhat opaque with yellow fibrinous plaques. Dougherty (1961) described in detail the pathology of *S. typhi-murium* and *S. enteritidis* infection in the White Pekin duckling. Gross enlargement of the liver with or without focal necrosis, formation of caseous plugs in the ceca, and enlargement and mottling of the rectum were the predominant lesions. Pericarditis, epicarditis, and myocarditis were found. Central nervous lesions consisted of thickened meninges in about one third of the typical salmonellosis cases confirmed by bacteriological studies. Price *et al.* (1962), in necropsy studies of ducklings infected with Salmonella, found necrotic foci in the liver, cheesy plugs in the ceca, impaction of the rectum, and blanching of the kidneys. Rasmussen (1962) described the pathology of arthritis in slaughter ducks due predominantly to *S. typhi-murium*. Among 827 arthritic ducks, *S. typhi-murium* was isolated from 12 per cent of the inflamed hock joints, 75 per cent of the knee joints, and 52 per cent of the hip joints. Perek and Rabinovitz (1957) found that at autopsy, goslings revealed pale flesh with some necrotic foci on the livers. Das *et al.* (1959) observed yellowish-green fibrinous deposits in the oral cavity and at the base of the tongue and on the upper palate of pigeons infected with *S. typhi-murium*.

Adult birds. Mature fowl generally exhibit no outward signs of the infection. Acute outbreaks in semimature and adult birds under natural conditions are rare. However, adults in infected flocks are often chronic carriers of paratyphoid organisms both in their internal organs and intestinal tracts. Wilson (1948) found that adult chickens may remain intestinal car-

riers of *S. typhi-murium* and *S. thompson* for periods up to 9 to 16 months.

Experimental paratyphoid infection of adult chickens and turkeys by either the parenteral or oral route of administration will result in an acute disease of short duration. Symptoms during the acute stage include loss of appetite, increased water consumption, diarrhea, dehydration, and general listlessness. Recovery is rapid in most cases, and death losses do not usually exceed 10 per cent.

Acutely infected adult fowl may reveal congested and swollen liver, spleen, and kidneys, hemorrhagic or necrotic enteritis, pericarditis, and peritonitis. Leg weakness in mature birds is not uncommon. Higgins *et al.* (1944) encountered a flock of 24-week-old turkeys infected with paratyphoid in which 10 per cent of the birds were so severely affected with an arthritic condition that they were unsuitable for marketing. Chaplin and Hamilton (1957) reported a synovitis in turkeys, similar to that ascribed to intravenous staphylococcal inoculation, following intravenous inoculation of broth cultures of *S. thompson*.

In an infected environment under natural conditions the disease is constantly transmitted among adults through consumption of the organisms; however, the period that the birds are infected may be transient. The degree to which adult chickens and turkeys become chronic carriers is dependent upon the number of organisms to which they are exposed, the virulence of the strains, and the general condition of the infected individuals.

Chronic adult carriers of paratyphoid infections are often submitted for necropsy as reactors to serological tests for pullorum disease or *S. typhi-murium* infection. Emaciation, necrotic ulcers in the intestines, enlarged liver, spleen or kidneys, nodules on the heart, and distorted ovules may occasionally be noted. Pathological changes in ovarian tissues as a result of paratyphoid infection are not as distinctive or common as observed in the case of chronic carriers of pullorum disease.

Chronically infected adults frequently exhibit no lesions. This is particularly true of intestinal carriers.

DIAGNOSIS

Clinical observations and necropsy findings may be suggestive of paratyphoid infection when a supportive history is available, and may permit one to reach a tentative diagnosis as a basis for early treatment or control recommendations. However, the final diagnosis of paratyphoid infections is dependent on the isolation and identification of the causative organisms. Procedures for this purpose require approximately 48 to 72 hours. The practicing veterinarian must usually rely on the state animal diagnostic laboratory for this service.

Biochemically and antigenically the members of the *Salmonella* genus are closely related not only to each other, but also to many other groups of the family *Enterobacteriaceae*, such as the Arizona and *Proteus* spp. Accurate identification of Salmonella and their differentiation from other closely related organisms often require detailed laboratory study. Specialized serological procedures necessary for the final identification of Salmonella types have been discussed under the section on Serology.

The bacteriologist or veterinarian engaged in the isolation of Salmonella cultures from poultry must be thoroughly familiar with the application of standard procedures for the culture and identification of members of the family *Enterobacteriaceae*. The manuals of Kauffmann (1950) and Edwards and Ewing (1962) on this subject are excellent laboratory references.

Serological tests have been applied only on a limited scale for the diagnosis of paratyphoid infections of poultry. This subject will be discussed in more detail in the section on Prevention and Control. Antigens used to test for paratyphoid infections may react positively to the serum of birds infected with pullorum disease, fowl typhoid, the Arizona, and other organisms. Bac-teriological examination must often be utilized to determine the presence of paratyphoid infections in reacting birds, especially when the reactions observed are of a suspicious nature. Thus, serological testing for paratyphoid should be regarded as a supplemental rather than a definitive diagnostic procedure.

In acute cases of paratyphoid infection in young birds, cultures should be prepared directly from the liver, spleen, heart blood, lungs, duodenum, and ceca. Additional organs or other portions of the intestinal tract may also be cultured if indicated. Mallmann *et al.* (1942), Poscll (1942), Hinshaw and McNeil (1943a), Gibbons and Moore (1946), Gauger and Greaves (1946c, 1947), and Chase (1947) have emphasized the importance of bacteriological examination of intestinal contents in attempting to determine the presence of paratyphoid infections in chicks and poults.

The culture of cloacal swabs taken from living birds has been studied as a diagnostic procedure for paratyphoid infection; however, this method is unreliable since fecal excretion of the organisms may be intermittent and it is impractical for use on a large scale (Wilson, 1948). Beattie (1960) indicated that fecal tests are not sufficiently reliable and, therefore, have limited application. These procedures may be employed, however, as a general measure in the detection of supply flocks that may contain Salmonella carriers. The bacteriological examination of yolk material from embryos dying between days 19 and 21 proved a practical method of detecting carrier flocks in Great Britain. Ellemann (1960) cultured 2,835 cloacal swabs from both chicks and adult hens in Sweden. Nine of the samples examined were found to contain 4 types of Salmonella organisms, and it was suggested that these were introduced into poultry flocks through contaminated imported feeds. Bierer and Vickers (1960b) examined cloacal swabs from nitrofuran-medicated, experimentally infected poults at 8 weeks of age. Swabs positive for *S. ty-*

phi-murium ranged from 0–33 per cent in the medicated birds and from 50–60 per cent in the nonmedicated control groups.

Cultures taken directly from fresh organs of diseased birds are made on beef-infusion agar slants or plates, which are incubated for 24 hours at 37° C. Intestinal cultures are subjected to selective enrichment in liquid broths after which they are streaked on selective agar plates. This technique is also useful in the bacteriological examination of specimens that are in a state of decomposition.

There are a large number of liquid enrichment broths and selective agars that have been recommended for the isolation of Salmonella cultures from fecal specimens. These have been reviewed by Edwards and Ewing (1962). Several media have been established, through practice, as most universally useful in the diagnosis of paratyphoid infections of poultry. For initial enrichment of fecal cultures most laboratories employ either tetrathionate broth with 1 to 100,000 brilliant green and 5 per cent bile, or selenite broth. Some laboratories use both of these media, which are available commercially in dehydrated form, in the examination of each fecal specimen. About 1 gram of fecal material is used to inoculate each 10 to 15 cc. of selective broth. Moran (1959b) reviewed procedures for the isolation, identification, and differentiation of Salmonella and Arizona strains. She strongly recommended the use of Kauffmann's tetrathionate enrichment medium, containing bile and brilliant green, and Kauffmann's brilliant-green agar for the isolation of Salmonella and Arizona cultures from intestinal contents. Stokes and Osborne (1955) and Osborne and Stokes (1955) developed a modified selenite brilliant-green medium for the isolation of Salmonella. This medium, through the addition of 0.05 per cent sodium sulfapyridine, was found to be sufficiently selective and sensitive to permit isolation of Salmonella, even when only one viable cell was present in 100 grams of commercial, naturally contaminated dried egg. Galton et al. (1952) reported that the addition of 0.125 mg. of sodium sulfathiazole to each 100 cc. of tetrathionate broth suppressed the multiplication of Proteus organisms. Jeffries (1959) found that tetrathionate containing 40 micrograms of novobiocin per milliliter of medium has proved better than plain tetrathionate as an enrichment medium for the isolation of Salmonella. The novobiocin was found to be very inhibitory for Proteus organisms. Galton (1961) reported the addition of 6 milliliters of a 10 per cent solution of Tergitol No. 7 to each 100 milliliters of tetrathionate brilliant-green broth for the isolation of Salmonella from animal feed products.

Enrichment broths are incubated for 18 to 24 hours at 37° C. before streaking on selective solid media. The most widely employed selective plating agars include SS agar, MacConkey agar, brilliant-green agar (Kauffmann, 1950), and desoxycholate citrate-lactose-sucrose agar. There is some variation in preferences for these plating media among various diagnostic laboratories. Edwards et al. (1948c) stated that the most satisfactory method for the examination of intestinal material from fowl for paratyphoid organisms is the use of tetrathionate brilliant-green broth plated after 24 hours' incubation on the brilliant-green agar of Kauffmann (1950).

Bloom et al. (1958), in culture studies of sewage, found a selenite brilliant-green enrichment medium to be superior to tetrathionate broth in the isolation of Salmonella. Dixon (1961) found that plating of selenite F enrichment cultures on a brilliant green MacConkey agar after 6 hours incubation at 43° C. added significantly to the number of positive isolations of Salmonella. Yamamoto et al. (1961b) did not find the number of Salmonella in turkey feces to change appreciably when samples were held 21 to 48 hours at 4° C. Ringer's solution and Hajna's preservative were effective as suspending media to maintain the organisms. Selenite-cystine broth enrichment with subsequent plating on brilliant-green agar gave the most favorable results in the isolation of *S. typhi-murium*

from infected tissues and other material.

Methods for the bacteriological examination of reactors to serological tests for paratyphoid infections are identical to those used in the laboratory examination of reactors to the pullorum test. Jungherr et al. (1950) outlined procedures for the bacteriological examination of reactors to serological tests for pullorum disease. Yamamoto et al. (1962) outlined cultural procedures for the examination of turkeys reacting to serological tests for S. typhimurium. Standard procedures for the isolation of Salmonella from poultry vaccines of egg-embryo origin have also been described (Anon., 1954).

Because of the interest in Salmonella organisms occurring in poultry feedstuffs, recommended procedures for the isolation of Salmonella organisms from animal feeds and meat by-products have been developed and published (Anon., 1962). Hobbs (1963) discussed in detail techniques for the isolation of Salmonella organisms from eggs and egg products. Papadakis (1960) developed a dulcitol-sucrose-salicin-iron-urea agar for the differential diagnosis of Salmonella, Proteus, and Arizona cultures. Since 1951 the Research Committee of the North Central Regional Poultry Disease Conference has conducted a comparative study of various culture media and techniques for the recovery of Salmonella from adult birds submitted for bacteriological examination as reactors to blood tests. The results of this study were published in the 1952 and 1953 Proceedings of the Conference. At the 1954 Conference the results of various surveys were summarized, and this information was used as a basis for the standard procedures for the examination of reactors to Salmonella serological tests which are published in the National Poultry and Turkey Improvement Plans and Auxiliary Provisions (Anon., 1963b).

The tissues most frequently selected for culture from serological reactors are the heart, liver, lungs, gallbladder, ovary, testes, oviduct, pancreas, and spleen. A composite sample of the above organs may be triturated in a sterile mortar, ground in a blender, or the organs may be processed individually. Nutrient broth is usually used as a diluent, and the suspensions are inoculated in 10 cc. aliquots per 100 cc. of selective broths such as tetrathionate brilliant-green, selenite, and a noninhibitory nutrient broth. Selective plating media are streaked from the broths after 18–24 hours at 37° C. If suspicious colonies are not observed on the plates after 24 hours' incubation, the enrichment broths may be restreaked on solid media. Intestinal cultures from reactors are prepared as described previously for young birds.

All selective plating media are examined after 24 hours' incubation at 37° C. for colonies typical of Salmonella. Suspected colonies are picked by touching the tip of a platinum needle to the center of the colony and transferred by stab and streak to the triple sugar iron (TSI) agar slants. The inoculated tubes are incubated for 24 hours at 37° C. Slants which have a typical acid butt (yellow) with or without gas and an alkaline slant (red) are selected for further study as possible Salmonella cultures. Hydrogen sulfide production is indicated by blackening of the agar due to iron sulfide precipitation. Sieburth (1957b) reported the development of a lactose urea agar in combination with a mannitol semisolid agar for the differentiation of Salmonella from other closely related enteric organisms. Ewing et al. (1960) described the deamination and carboxylase reactions of Salmonella and Arizona strains and pointed out their value in taxonomy of the groups.

In order to eliminate Proteus spp., it is advantageous to test the suspected Salmonella cultures for urease production. The urea agar of Christensen (1916), which permits a reading in 6–8 hours, or the rapid urease test of Stuart et al. (1945) may be used.

Those cultures that are negative to the urease test should be transferred to the following media for final identification: dextrose, lactose, sucrose, mannitol, maltose, dulcitol, malonate, and salicin; peptone medium for indol production; semi-

TABLE 9 3

TYPICAL REACTIONS OF PARATYPHOID, ARIZONA, AND CITROBACTER
CULTURES ON DIAGNOSTIC MEDIA*

Media	Paratyphoid	Arizona	Citrobacter
Dextrose..	+	+	+
Lactose. .	—	+ or x	usually +
Mannitol..	+	+	+
Maltose .	+	+	+
Dulcitol...	usually +	+	usually +
Malonate .	—	+	—
KCN. ..	—	—	+
Ninhydrin .	+	+	—
Urease.. .	—	—	—
Indol.. .	—	usually —	usually —
Gelatin	—	(+)	usually —
Motility	+	+	+

*+, positive in 1–2 days, acid and gas in carbohydrate media; —, negative; x, late or
irregularly positive in 7–10 days, (+), gelatin liquefied slowly.

solid medium for motility; and gelatin. A
Gram stain of each culture should also be
prepared. Checking of each culture with a
polyvalent Salmonella typing serum is
helpful in identification.

Typical reactions of paratyphoids, Ari-
zona, and Citrobacter on diagnostic media
are listed in Table 9.3.

Garside et al. (1960) used phage-typing
of S. typhi-murium in distinguishing a sin-
gle strain of this organism in experimental
studies of antibiotic resistance. Anderson
and Wilson (1961) have described a bac-
teriophage-typing scheme for S. typhi-mu-
rium strains of animal origin. They sug-
gested that the more extensive veterinary
use of this procedure will aid in tracing the
distribution of particular types of S. typhi-
murium infection in animals. Marthedal
(1962) used a biochemical and serological
scheme for classifying S. typhi-murium
strains of avian origin in Denmark.

TREATMENT

Medicinal therapeutic measures may be
employed to reduce mortality in acute
outbreaks of paratyphoid infections and
to aid in preventing the development and
spread of the disease. All such measures
have the disadvantage of being incapable
of eliminating the infection from treated
birds. The demonstration by Yurchenco
et al. (1953) that certain of the furan ring
type of chemicals (nitrofurans) have

marked activity against Gram-negative
bacteria opened a new field in the ther-
apy of Salmonella infections of poultry.
Wolfgang (1958) reviewed recent progress
in nitrofuran research.

The use of chemical therapeutic meas-
ures for paratyphoid infections in domes-
tic poultry flocks should be restricted to
flocks intended for market use. Young
birds that have undergone an acute out-
break of paratyphoid infection, even when
drug therapy is used, are not a safe source
of future breeding stock. Birds that have
received treatment may remain carriers of
the organisms as therapeutic measures are
not capable of completely eliminating the
infection. It should always be remembered
that while advances have been made in the
prevention of paratyphoid and reduction
of losses through treatment, therapeutic
measures are of little value in any long-
range program to eliminate paratyphoid
infections from poultry flocks.

Individual or flock therapy may be in-
dicated in acute outbreaks of paratyphoid
infections among birds maintained in zo-
ological parks, for treating household pets
such as parakeets or canaries (Burkhart et
al., 1962), and for flocks of wild birds main-
tained on refuges (Lucas, 1956 and Jones,
1959).

The sulfonamide drugs have been dem-
onstrated to have some effect in reducing
the mortality from paratyphoid infections.

Sulfamethazine and sulfamerazine have been most widely used and are usually administered as feed additives. Feeding of the sulfonamides at the therapeutic level should be intermittent to prevent the development of toxicity from the treatment. Turkey poults are particularly susceptible to the toxic effects of the drugs. Soluble forms of some of the sulfonamide drugs are available for addition to the drinking water. This method of administration is advantageous when the birds do not consume feed in quantity. Sulfonamide-resistant bacterial forms may develop from continued treatment.

Clark (1916) reported that sulfamerazine in the mash at a level of 0.3 per cent or higher was effective as a prophylactic measure against paratyphoid infections of chicks and poults. Peterson (1947) encountered a serious outbreak of S. typhi-murium infection in which sulfamerazine at a 0.5 per cent concentration in the mash was ineffective. Eveleth et al. (1947) reported that 0.4 per cent sodium sulfamerazine in the drinking water or 0.5 per cent sulfamerazine in the mash was found useful in reducing death losses from paratyphoid infections in poults and chicks. Pomeroy et al. (1948) reported that sulfamethazine, sulfadiazine, and sulfamerazine reduced death losses from S. typhi-murium infection in chicks 50 per cent; however, the drugs proved to be only one-half as effective when fed to poults. Price et al. (1962) used soluble sulfamethazine in the drinking water at the rate of 1 ounce per gallon of water for 2 days in the treatment of salmonellosis of ducklings.

The antibiotics have not been extensively used in the treatment of paratyphoid infections of poultry. In general, they have proved less effective than other medicinal treatments that are available. Lukas and Bradford (1951) reported that Terramycin and chlortetracycline (Aureomycin) were the drugs of choice in controlling paratyphoid outbreaks in poults. The effective concentrations were 100 p.p.m. of soluble Terramycin in the water and 250 grams of Terramycin or chlortetracycline per ton of

feed. The duration of treatment was determined by the flock response. Other flocks that received sulfonamide therapy had a higher percentage of losses and stunted birds than did the antibiotic-treated group. Garside et al. (1960) found that chlortetracycline gave a measure of protection against artificial infection with S. typhi-murium in chicks, the mortality rate in antibiotic-fed chicks being approximately one-half that in chicks not receiving the antibiotic.

Thiocymetin (Shaffer et al., 1954) and synnematin B (Olson and Jennings, 1954), two newer antibiotics, have shown encouraging results in experimental applications for the treatment of avian salmonellosis.

Ravioli and Orfei (1953) found Chloromycetin and Terramycin to change the fermentative properties of Salmonella isolated from treated birds. McCarty (1953) reported neomycin to be beneficial in the treatment of S. derby infection in geese, and Schoop and Moser (1954) reported that streptomycin was used with some success in the therapy of pigeons infected with S. typhi-murium.

Mitrovic et al. (1961) tested the sensitivity of 20 Salmonella types in vitro to a new chemotherapeutic agent, 3, 5-dinitrobenzamide. The organisms were sensitive to the drug at levels of 1.0, 5.0, and 10.0 mg. per test disc. In vivo studies revealed that the compound reduced mortality in S. typhi-murium-infected poults when fed either 3 days before, simultaneously with, or 1 day after infection.

Furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone], one of the nitrofuran derivatives of furfural, has been found to be effective in reducing mortality in acute outbreaks of several types of paratyphoid infections. Subtherapeutic levels of the drug are recommended to decrease the development and spread of paratyphoid infections. Furazolidone is marketed under the trade name, nf-180.

In recommended concentrations, furazolidone has a low toxicity for host tissues and acts by interrupting the enzymatic metabolic processes of the microbial cell.

drug was found to be effective in lowering death losses from the infection and also in increasing the body weights of birds being treated.

Belding and Mayer (1958) reported that furazolidone added to the mash of poults at the level of 0.011 per cent for 2 weeks followed by a 0.0055 per cent level for 3 weeks reduced the mortality due to *S. san diego* infection by 50 per cent. Treatment was started at the time the poults were experimentally infected. Aureomycin and sulfamethazine were found to have no appreciable effect in reducing mortality due to the infection. Treatment with either Aureomycin and sulfamethazine or furazolidone reduced the number of infected carriers.

Bierer and Vickers (1960a) studied the effect of 4 nitrofurans on *S. typhi-murium* infection induced experimentally in poults. Results were inconclusive, and none of the nitrofurans used was completely successful in eliminating carriers of *S. typhi-murium* from the infected groups. Bierer and Vickers (1960b) found that furazolidone in the feed or soluble nitrofurans in the drinking water drastically reduced mortality from experimentally induced *S. typhi-murium* and *S. heidelberg* infection in poults. The soluble nitrofurans used were NF-248 and solubilized furaltadone at the level of 0.0066 and 0.0264 per cent, respectively, in the drinking water. These drugs did not depress water consumption. Solubilized furaltadone would have been the nitrofuran of choice if it was not feasible or desirable to medicate through the feed. Bierer *et al.* (1961c), in further studies of water-soluble furaltadone medication, found that the drug administered at the rate of 0.25 gm/gal of water resulted in a marked reduction in mortality from *S. typhi-murium* infection in poults. Using furaltadone prophylactically in the drinking water at the levels of 0.25, 0.5, and 1 gm/gal, Bierer and Barnett (1962b) found the drug to reduce mortality from *S. typhi-murium* infection in turkey poults from 70–80 per cent to 20–30 per cent. Bierer (1963) reported that both 0.011 per cent and 0.0165 per cent nihydrazone (Nidra-

fur) reduced mortality due to *S. typhi-murium* infection in turkey poults. However, this drug seems to retard growth at effective therapeutic levels in turkeys.

Lucas (1956) reported that furazolidone at the level of 0.011 per cent in the feed was used successfully to control an outbreak of *S. typhi-murium* and *S. anatum* infections in wild mallard ducks. Tablets of the drug were also found to be effective in individual treatment at a dosage of 50 mg. daily. Agglutination tests using *S. anatum* and *S. typhi-murium* antigens revealed the presence of reactors after treatment. Truscott (1956) found that a level of 0.011 per cent furazolidone in the feed did not alleviate an outbreak of salmonellosis among ducklings. However, when the concentration was increased to 0.0165 per cent and strict sanitation measures instituted in the brooding pens, the infection was brought under control. Price *et al.* (1962) indicated that treatment of paratyphoid-infected ducklings was usually limited to rigid culling. Sulfamethazine in the drinking water was recommended as medication in more serious outbreaks.

Jones (1959) recommended furazolidone as the drug of choice in the treatment of Salmonella and Arizona infections of game birds. Francis *et al.* (1960) reported that pheasants and quails may effectively be treated for salmonellosis with 0.011–0.022 per cent furazolidone for 7–10 days followed by 0.0055 per cent for another 7–10 days.

Keymer (1959) recommended a compound consisting of nitrofurazone and furazolidone added to the drinking water in the treatment of salmonellosis of canaries and other passerine birds. Treated birds may remain carriers of the organisms. Burkhart *et al.* (1962) reported treatment of parakeets and canaries with several watersoluble nitrofurans following experimental paratyphoid infections. Furaltadone (NF-260) and a second nitrofuran (NF-248) in a 6 per cent propylene carbonate solution, administered in the drinking water, controlled *S. typhi-murium* infection in parakeets when used for 2 weeks at the level of

preventing cell multiplication (Paul, 1956). The drug has a low solubility which permits the extended maintenance of a rather high concentration in the intestinal tract of treated birds. Smith (1955) demonstrated, however, that furazolidone is usually totally absorbed near the middle of the avian intestinal tract. The drug is generally administered at a concentration of 0 0055 per cent (50 grams per ton) fed in the mash on a continuous basis as a preventive of paratyphoid infections in birds over 2 weeks of age or at the level of 0.011 per cent (100 grams per ton) as a preventive in birds during the first 2 weeks after hatching. A dosage schedule of 0.011 per cent to 0.022 per cent (200 grams per ton) in the mash fed for 2 weeks is recommended for treatment of acute outbreaks of paratyphoid infections The drug is usually added at the required level by the feed manufacturer.

Paratyphoid organisms are apparently more resistant than S pullorum and S. gallinarum to the inhibitory action of furazolidone both in laboratory tests and in field therapy trials. Smith (1955) during in vitro sensitivity studies found that the 9 strains of S. typhi-murium included in his tests were 2 to 4 times as resistant to furazolidone as was S. pullorum. Harwood (1956) indicated that furazolidone only rarely eliminated paratyphoids from infected birds although clinical response in reducing mortality from the disease was excellent.

Smith (1955) reported that furazolidone fed at a level of 0.04 per cent in the mash continually for 10 days was very effective in reducing the mortality associated with experimental S. typhi-murium infection in poults and chicks. It was found that a high proportion of treated birds become carriers of S. typhi-murium even when treatment was started 3 days before infection. The organisms were more frequently recovered from the feces than from the organs of experimentally infected birds Repeating the treatment or increasing the concentration of furazolidone was not found to decrease the carrier rate.

Wilson (1955) conducted experiments to test the efficacy of furazolidone in treating S. typhi-murium and S. thompson infections in chicks. Both 0.02 per cent and 0.04 per cent levels of the drug were used in these experiments. Results were inconclusive as the disease did not develop to any degree in control groups that received no treatment. Harwood (1956) suggested that a combination treatment of furazolidone and sulfonamide may prove useful in reducing losses in extremely severe paratyphoid epizootics of poultry.

Sieburth (1957a) studied the effect of 0.011 per cent furazolidone on the mortality rate of chicks experimentally infected with S. typhi-murium at 1 day of age. Uninfected controls and furazolidone-treated groups both experienced 7 per cent mortality while the infected groups that received no treatment experienced a mortality of 27 per cent. However, 100 per cent of the intestinal cultures and a majority of the organ pools from the surviving chicks contained detectable numbers of S. typhi-murium. The drug also had an inhibitory effect on the development of S. typhi-murium agglutinins detectable by a conventional agglutination test. Agglutinins were demonstrable, however, through the use of an indirect hemagglutination test.

Beattie (1960) reported that he used furazolidone with excellent results in the treatment of experimental S. thompson infection in chicks. Lannek et al. (1962) reported studies of the nitrofurazone compound, Tiafur, in the treatment of experimental and spontaneous Salmonella infections in chicks. Mortality was considerably reduced and clinical symptoms were markedly suppressed by a level of 0.1 per cent of Tiafur in the feed. A long-time treatment with small doses (0.01 and 0.04 per cent) gave more chicks the chance to survive without eliminating infection, and consequently left more carriers. Bierer and Barnett (1962a) studied the use of the nitrofuran feed additive, Nihydrazone (Nidrafur), at the 0.011 and 0.022 per cent levels, in the therapy of incubator-induced S. typhi-murium infection in chicks. The

drug was found to be effective in lowering death losses from the infection and also in increasing the body weights of birds being treated.

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300 mg. total nitrofuran per liter of water. This combination at 150 and 200 mg. levels gave 100 per cent protection against *S. typhi-murium* infection in canaries. It was emphasized that increased water consumption during paratyphoid infections makes the use of water-soluble nitrofurans preferable. Also pet birds like parakeets and canaries consume seeds which makes it impossible to administer the drugs in the feed.

Bacterins, serums, or similar biologicals are not effective in either the prevention or treatment of paratyphoid infections of poultry.

PREVENTION AND CONTROL

Adult intestinal carriers serve as the chief source of paratyphoid infections in most species of poultry. Fecal contamination of hatching eggs by the chronic carrier or from an infected environment is the means by which the infections are generally introduced into the incubator and subsequently into the brooder. Because of the wide natural distribution of Salmonella organisms, the numerous antigenic types, and the lack of adequate methods to detect carriers among adult flocks, *hatchery and flock sanitation is the most important factor in the prevention and control of paratyphoid infections of poultry.*

Flocks that experience acute outbreaks of paratyphoid with high losses at a young age, or those that are known to carry the infection at any age, should not be used as a source of eggs for hatching purposes. Early disposal of such flocks is usually the most desirable program to follow. Agglutination tests have been employed to detect infected flocks; however, procedures for this purpose have not been developed to the degree of accuracy of serological tests for the detection of pullorum disease and fowl typhoid. For prevention of the infections, replacement stock and hatching eggs should be obtained from a source that is known to be paratyphoid free. The birds should be maintained at all times in an environment where exposure to the organisms is kept

at a minimum. Goetz (1962) discussed the California program used for the control of *S. typhi-murium* and Arizona infections in turkeys. The program is based on the following principles: (1) Stocking the breeding flock from a known clean source; (2) bacteriological sampling of all poult mortality up to 21 days of age; (3) tube agglutination testing of all potential breeding stock with *S. pullorum*, *S. typhi-murium*, and Arizona antigens; and (4) routine bacteriological examination of samples of 10-day-dead embryos from the hatchery.

Paratyphoid infections, when encountered in valuable breeding stock, constitute a particular problem. Special measures are necessary in such cases, and eradication efforts often extend over a period of several years involving considerable financial loss. The most practical way to control the disease among birds in the pet or bird store is by means of depopulation and thorough cleaning and disinfection of the premises. A critical examination should be made of the source of supply for replacements in order to prevent recurrent infection.

An effective program for the prevention and control of paratyphoid infections of poultry should give consideration to the following measures:

1. Hatchery and egg sanitation. The use of only clean eggs for hatching purposes will lessen the chances of introducing the infection into the incubator through fecal contamination. Adequate numbers of nests and clean laying houses should be provided. Eggs should be collected at frequent intervals, fumigated, and stored in a cool place for as short a period as possible before setting. Cleaned and disinfected containers should be used in collecting the eggs, and the person making the collections should be certain that he does not serve as a source of contamination from organisms that may be present on his clothing or hands. Dirty eggs should not be used for hatching purposes, and should be collected in a separate container from hatching eggs.

Racks or crates used for storing eggs

should be new or properly cleaned and disinfected. Contact of the eggs should be kept at a minimum during storage since one egg or may serve as a source of contamination for many others. Maclaury and Moran (1959) stressed the fact that if warm, freshly laid eggs do not come in contact with contaminated soil or fecal material while they are cooling, the chances of their being infected with Salmonella organisms are greatly decreased. If it is necessary to transport eggs before setting, new crates are most desirable for this purpose. Dirty crates should never be used. Pomeroy (1958) has pointed out that contaminated eggshells may serve as an important means of introducing Salmonella and Arizona organisms into a hatchery. Attention to sanitation on the farm at the supply flock level to obtain Salmonella-free hatching eggs is an essential part of a paratyphoid control program.

Dipping or washing dirty eggs before setting is not recommended as this practice may contribute to the penetration of the eggshell by Salmonella organisms that are present on the surface. Quaternary ammonium compounds, formalin, sodium hydroxide, and sodium orthophenylphenate have been studied for this purpose (Wilson, 1948); however, most investigators are not convinced that this is a practical or effective procedure for the prevention of salmonellosis. Frank and Wright (1956) reported that 0.5 per cent sodium hydroxide was effective within 5 minutes in disinfecting pieces of eggshell infected with S. typhi-murium. However, dipping whole eggs in sodium hydroxide at concentrations up to 2 per cent for 5 minutes failed to prevent penetration of the eggshell by the organism both at incubator and room temperature. It was felt that penetration of the shells may have occurred before the disinfectant had a chance to act or that the organism was protected by some physical property related to the pores of the shell.

Mundt and Tugwell (1958) and Beattie (1960) have suggested dipping eggs in germicides as a reliable method of control. Bierer et al. (1961a) found that none of 24 chemical egg-washing compounds was ef-fective in killing S. typhi-murium when used at 2.5–4 times manufacturer's recommendations. Quaternary ammonium compounds were found to be entirely unsatisfactory. One per cent zinc sulfate was found to be superior to the other chemicals used from the standpoint of causticity, toxicity, odor, and staining. Bierer et al. (1961b) used 8 commercial compounds advertised as detergents or detergent-sanitizers in egg-washing experiments. The compounds were from 52 to 92 per cent efficient in removing or destroying artificially induced S. typhi-murium eggshell contamination. Bierer and Barnett (1961) demonstrated that egg-wash germicides should be tested at 38° C. or lower to avoid the germicidal effect of the water itself in experimental studies.

Early fumigation of the eggs with formaldehyde gas has been found to be very effective in the prevention of egg-borne paratyphoid infections. Wilson (1949) stated that fumigation of eggs with formaldehyde gas is probably the most important factor in paratyphoid prevention. Recommendations on the application of formaldehyde gas as an incubator fumigant for the prevention of pullorum disease are discussed in the chapters Principles of Disease Prevention and Pullorum Disease.

Egg fumigation for the prevention of pullorum disease is generally recommended for application toward the end of the incubation period. Since paratyphoid infections, unlike pullorum disease, are usually transmitted through penetration of the eggshell by organisms on its surface, early fumigation is required. Marcellus et al. (1939) have demonstrated that the embryos are most susceptible to injury from formaldehyde gas during the 24th and 96th hours. Fumigation during this period is to be avoided.

Lancaster (1962) reviewed information relating to formaldehyde fumigation of eggs with high levels of the gas. It was pointed out that earlier studies on the period of maximum susceptibility of embryos are not necessarily applicable when higher levels of the fumigant are used. With these

larger amounts the period of embryonic susceptibility appeared to be prolonged from the second to the sixth or even eighth day of incubation. Since Harry and Binstead (1961) were able to demonstrate that hatchability may be adversely affected when embryos are exposed to high levels of formaldehyde gas between the third and ninth day of incubation, preincubation fumigation as a standard procedure was highly recommended. The degree to which hatchability was affected was influenced by the particular flock involved and the preincubational storage period.

Fumigation of hatching eggs as soon as possible after collection is highly recommended. A room or cabinet sized proportionally to the number of eggs should be provided. Fans can be used to circulate and exhaust the gas from enclosed areas. Eggs for fumigation should be placed on racks which will permit good air circulation. Formaldehyde gas is provided by mixing 0.6 gram of potassium permanganate with 1.2 milliliters of formalin (37.5 per cent) for each cubic foot of space in the cabinet or room. The chemicals should be mixed in an earthenware or enamelware container having a capacity of at least 10 times the volume of the total ingredients. The gas should be circulated within the enclosure for 20 minutes, then expelled. Humidity for this type of fumigation is not critical, but the temperature should be around 70° F. Extra humidity may be provided in dry weather.

When preincubation fumigation is impossible, eggs should be fumigated as soon as possible (preferably within 12 hours) after setting. After allowing temperature and humidity to regain normal operating levels, formaldehyde gas should be released into the incubator. For each cubic foot in the incubator, 0.4 gram of potassium permanganate and 0.8 milliliter of formalin (37.5 per cent) are used. Doors and vents should be closed with circulating fan operating for a 20-minute period of fumigation with normal operating temperature and humidity. After fumigation, vents should be opened to the normal operating

positions to release the gas (Anon., 1963b). An illustrated bulletin in which the importance of preincubation fumigation of turkey hatching eggs is discussed has been published by the California Department of Agriculture (Stover, 1960).

Buxton and Gordon (1947), Wilson (1948, 1949, 1951), Clarenburg and Roepke (1952), Lancaster and Crabb (1953a, b), Lancaster et al. (1954), Harry (1954), Clarenburg and Romijn (1954), and Frank and Wright (1955), have studied the use of formaldehyde gas in the fumigation of eggs and incubators contaminated with paratyphoid organisms. Buxton and Gordon (1947) and Wilson (1949) have recommended that fumigation for the destruction of paratyphoid organisms on the shell surface should be done before incubation. Penetration of the shell was found to proceed far more rapidly in the warm, humid atmosphere of the incubator, and usually to occur during the first week of incubation.

Wilson (1951) recommended the fumigation of eggs for a period of 30 minutes with formaldehyde gas produced by the addition of 150 ml. of formalin to 100 grams of potassium permanganate per 100 cubic feet of incubator space. This concentration of the gas was invariably lethal for S. typhi-murium on eggshells and on down, and normally had no adverse effect on hatchability if certain precautions as to the time of application were taken. Humidity was not a factor as this treatment was found to be effective even under ordinary atmospheric conditions. The high concentration of the gas was recommended particularly following an actual outbreak of paratyphoid infection.

Frank and Wright (1955) studied the susceptibility of 19 Salmonella serotypes to formaldehyde fumigation. Test organisms were placed on pieces of eggshell and string. Fumigation with 150 milliliters of formalin and 75 grams of potassium permanganate per 100 cubic feet of incubator space killed all organisms in 20 minutes, while a number survived 10 minutes fumigation. When 100 milliliters of formalin

and 50 grams of potassium permanganate per 100 cubic feet were used, 30 minutes were required to kill all organisms. *S. pullorum* was found to be more susceptible than the paratyphoids to the lethal effects of the formaldehyde gas.

For routine fumigation of eggs to destroy paratyphoid organisms Wilson (1951) recommended the use of 75 ml. of formalin and 50 grams of potassium permanganate per 100 cubic feet of incubator space with a dry bulb reading of 100° F. and wet bulb reading of 90° F. A suitable time for fumigation was found to be 6–8 hours after the eggs were set. A later fumigation toward the end of the incubation period is of further value in the prevention of incubator spread of the disease. Harry (1954) found that the removal of residual formaldehyde remaining after fumigation could be accomplished by adding 33 per cent ammonia solution to the incubator in a volume equal to one-half that of the formalin used. This limited the escape of irritant concentrations of formaldehyde from the incubators.

When an air-borne infection of Salmonella organisms has become established in a hatchery it may be necessary to fumigate the entire hatchery with high levels of formaldehyde gas to destroy the organisms. Close attention should also be given to contaminated egg sources which are usually responsible for introduction of the infection into the hatchery. Blood testing and the culture of eggs from supply flocks are often useful adjuncts in locating the source of the infection.

Price *et al.* (1962) recommended that duck eggs be fumigated on the second day of incubation for a period of 15 minutes and prescribed the use of 1 ounce of formalin and one-half ounce of potassium permanganate to every 80 cubic feet of incubator space. Rasmussen (1962) also discussed hygienic measures to control the spread of duck salmonellosis.

After each hatch, incubators should be thoroughly cleaned of the debris of the hatch, washed with detergent and hot water, disinfected, and fumigated with a

bigh level of formaldehyde gas. At least 0.6 gram of potassium permanganate and 1.2 milliliters of formalin (37.5 per cent) per cubic foot of incubator space should be used with an exposure of 1 hour. Higher levels of the gas may be used if desired. General disinfection procedures for incubators and hatching rooms are discussed in Chapter 5.

Restrictions should be placed on hatchery personnel to insure that they do not introduce the infections into the hatchery from older fowl or from other animals with which they come in contact in their daily operations. It should be determined that no human carriers exist among the personnel. Visitors to the hatching area should be kept to a minimum. Boxes used to ship birds should be new, and trucks employed for transporting operations should be kept clean and be frequently disinfected. There should be a constant campaign to eliminate rats, mice, and flies in the vicinity of the hatchery.

2. Sanitation during the brooding period. For prevention of paratyphoid infections during brooding it is important that the young birds be constantly isolated from sources of the infection. Personnel that are in contact with older birds and with other animals should take precautions not to introduce the infection through droppings that may adhere to the shoes, clothing, or hands. It is a good practice under these conditions to wear overshoes that can be disinfected, and also coveralls that can be frequently changed. Bierer (1960) emphasized the importance of starting young turkey poults on cleaned and disinfected premises and possibly administering preventive medication in the initial feed and water in preventing paratyphoid infections.

All types of animals should be restricted from the brooding area. Feed and water containers should be situated where they cannot be contaminated by droppings and should be frequently cleaned and disinfected. Live steam is very effective for this purpose. A detailed sanitation program for the poultry industry has been outlined

(Anon., 1947) in which cresylic disinfectants are recommended. The standard dilution of cresylic disinfectant used is 4 fluid ounces to each gallon of water (about 3 per cent). Commercial lye, 2 per cent in water, is also a very effective disinfectant when used as a hot solution. Odorless disinfection is available through the use of sodium orthophenylphenate in an aqueous or detergent solution.

Young birds that die or are sick should be promptly submitted to the diagnostic laboratory for examination. It is the responsibility of the laboratory to conduct complete bacteriological examinations to detect any Salmonella that may be present. All Salmonella cultures isolated should be typed serologically and up-to-date and complete records maintained relative to the Salmonella types recovered from each flock within an area. This information is essential in guiding the use of specific antigens in serological tests for the disease in breeding flocks. Information derived from typing the organisms also aids in tracing the origin of disease outbreaks. Periodic summarizing of this information within each state would be most helpful to those engaged in paratyphoid control activities.

3. Flock sanitation. Rodents and various pests around the poultry yards serve as an important source of Salmonella organisms for semimature and adult flocks. Mice and rats may pass large numbers of the organisms in their excreta and are able to contaminate feed supplies and water, as well as litter and the poultry yards. It is hoped that a satisfactory sanitation code may be developed and adopted to insure that Salmonella organisms are not present in the feed which the flock consumes. This hazard must be removed before other control measures can be effectively applied. An active rodent eradication campaign is an essential part of the general Salmonella control program. Dogs, cats, sheep, cattle, horses, and swine should never have access to poultry operations.

Birds that have received therapy for paratyphoid infections should not be kept for breeding purposes. Neither should breeding flocks be treated for these infections and then maintained as a source of hatching eggs. Garside et al. (1960) found that a large number of carriers remained among groups of chicks receiving therapeutic levels of chlortetracycline. They emphasized that such a practice increases the problem of the control of salmonellosis in poultry. Goetz (1962) reported an incidence in which it was necessary to abandon turkey raising operations in one area because S. typhi-murium was indigenous in the wildlife of the area. The organism was isolated from gopher snakes, ground squirrels, and owls shot on the premises.

4. Serological testing. Procedures for the serological detection of adult carriers of paratyphoid infections have not been accepted or applied on the scale of those employed for the detection of pullorum disease and fowl typhoid. The tube agglutination test for S. typhi-murium has been most frequently used as a supplementary measure to other means of control or as a method for locating infected flocks. This procedure has been most widely applied in testing turkey breeding flocks.

Intestinal carriers may reveal no serological response to the agglutination test, and titers of birds that do react may fluctuate widely as reported by Pomeroy and Fenstermacher (1943), Buxton and Gordon (1947), and Lee (1957). Testing programs for paratyphoids are further complicated by the large number of antigenic types of the organism infecting poultry and the need for refinements in methods of producing antigens for the test.

Buxton (1958) noted that the agglutination test is of value in controlling salmonellosis of turkeys provided it is used in conjunction with hygienic measures to prevent reinfection. The agglutination test was found to give some idea of the rate of multiplication of the organism in the host and the carrier animal usually revealed a low titer. Bierer and Vickers (1960a) reported that in nitrofuran-medicated poults there was a definite suppression of agglutinin production to the extent that S. typhi-

murium experimentally-infected birds treated with nitrofurans did not react to any serological tests.

Specific antigens for the Salmonella type involved must be prepared in the laboratory. Laboratory personnel conducting the tests and interpreting the results must be thoroughly familiar with Salmonella serology and the variations that may arise during testing procedures. The history of each flock tested must be carefully considered. Representative reactors to the agglutination test should be submitted for complete bacteriological examination and laboratory confirmation of the presence of the infection.

The agglutination test has been applied in testing most species of fowl for paratyphoid. The use of the test has been sporadic in most areas, being applied as conditions warranted. In 1964 approximately 6 states in the United States were using the test on a large scale to detect *S. typhimurium* in turkey breeding flocks. At least 3 states (Minnesota, California, and Texas) have official rules and regulations including blood testing programs that have been adopted for the control of *S. typhi-murium* infection of turkeys. In a committee report (Anon., 1958a) it was suggested that if a designation or classification is needed for a flock under a voluntary *S. typhimurium* testing program, a classification such as "*S. typhi-murium* tested and no reactors found" should be considered. Pomeroy (1958) recommended that the typhimurium testing program of breeding flocks may be considered in areas where the disease is a problem. Serological tests in conjunction with bacteriological examination of cull chicks and poults will help to identify infected flocks. Müller (1957b) reported that in Denmark the selling of ducklings, goslings, and turkey poults hatched in incubators is allowed only when the hatching eggs originate from flocks in which no reactors to the *S. typhi-murium* and *S. enteritidis* tube agglutination tests have been found one month before the beginning of the hatching season.

Lee *et al.* (1936), Cherrington *et al.*

(1937), Pomeroy and Fenstermacher (1939), Hinshaw and McNeil (1943a, 1944a), Pomeroy (1944), McNeil and Hinshaw (1951), Delay *et al.* (1954), Pomeroy *et al.* (1957a), Ellis (1957), Lee (1957), Goetz (1962), and Yamamoto *et al.* (1962) have reported on the use of the agglutination test in turkey flocks. Hinshaw and McNeil (1943a) used a 1–25 dilution as a finding test and recommended that both a somatic (O) and flagellar (H) antigen be used to test each serum sample. Hinshaw and McNeil (1943b) used an H-type antigen to test turkeys for *S. newington* infection.

Delay *et al.* (1954) reported that 7,578 of 593,341 turkeys tested with *S. typhimurium* antigens by the tube agglutination method reacted in the diagnostic dilution of 1–25. Of this number, 4,486 were from flocks subsequently found to have *S. typhimurium* carriers. The O antigen was found to be most useful in detecting carriers, and birds revealing positive tests to both the O and H antigens were found most likely to yield *S. typhi-murium* on culture. They emphasized that a testing program for *S. typhi-murium* is most effective when it is possible to practice complete replacement of all flocks shown to harbor carriers of the organisms. Pomeroy *et al.* (1957a) also recommended that in a control program, based on the use of the agglutination test, reacting flocks should be disposed of, equipment and housing cleaned and disinfected, and replacement birds secured from a known clean source.

Belding and Mayer (1958) studied the use of the tube agglutination test to detect turkeys experimentally infected with *S. san-diego*. Only about one-third of the infected birds were detected by the test under the conditions of their experiment. Bierer and Vickers (1960a) found the rapid serum plate test using stained antigen for *S. typhimurium* to be superior to the tube agglutination test or the rapid whole-blood test in detecting turkeys demonstrated bacteriologically to be carriers of the organism. However, serological tests were not entirely effective in detecting the infection. Stover

(1961) pointed out that serological testing for *S. typhi-murium* in turkeys has helped to detect the infection in flocks that were subsequently disposed of or in which rigid sanitation including early heavy fumigation of eggs was applied. Yamamoto *et al.* (1961a) reported a positive correlation of 33.6 per cent between serological findings and isolation at 14 days after crop induced *S. typhi-murium* infection in adult turkeys. The percentage correlation had increased to 38.8 per cent at necropsy 35–44 days postinoculation. Yamamoto *et al.* (1962) found in Oregon that turkeys reacting as 3+ and 4+ to the agglutination test for *S. typhi-murium* had a higher isolation rate of the organism on culture. It was apparent, however, that a flock could not be considered infected on the basis of serology alone. Isolation rate on a flock or per-bird basis was optimum when 3 or 4 suspect birds were examined per flock. Serological testing was recommended merely as a part of the entire control program. Goetz (1962) described the role of the tube agglutination test for *S typhi murium* in the paratyphoid control program in California.

Schalm (1937), Buxton and Gordon (1947), Wilson (1948), Clarenburg and Romijn (1954), Gwatkin and Dzenis (1954), Gwatkin and Grinewitsch (1955a, b) and Sieburth (1957a, c) have reported the use of the agglutination test to detect carriers of paratyphoid infections in chicken flocks. Wilson (1948) indicated that the agglutination test is not likely to become a practical method for the detection of carriers of paratyphoid infections in chickens. Buxton and Gordon (1947) reported that in known infected flocks, the use of blood testing was believed to have merit as an adjunct to hygienic measures in the hatchery. Muller (1957a) used the agglutination test in examining 30,596 blood samples from ducks, geese, turkeys, and chickens from 1,356 flocks in the Danish islands. It was found that 26.4 per cent of the flocks contained reactors to *S. typhi-murium* and *S. enteritidis* antigens. Infection was confirmed by bacteriological cul-

ture in 41 of 361 reactors from 197 flocks. *S. typhi-murium* infected birds were found on one farm where the farmer and his family had experienced *S. typhi-murium* gastroenteritis.

Smyser and Van Roekel (1959), in the study of a supply flock to an institution where an outbreak of Salmonella food poisoning had occurred, serologically tested 5,050 chickens with the tube agglutination test for *S. typhi-murium*. Ninety-four reacting birds were detected, and 9 of the higher-titered reactors were cultured. *S. typhi-murium* was isolated from 2 birds. Müller (1961), reporting on the progress of the elimination of salmonellosis in poultry of the Danish islands, noted that more extensive serological testing programs should be conducted for *S. typhi-murium* infection.

Levine and Graham (1942) and Gordon and Garside (1944), among others, have reported on the use of paratyphoid antigens to test ducks. Lucas (1956) used furazolidone treatment and blood testing in the elimination of paratyphoid infection from mallard ducks. Perek and Rabinovitz (1957) concluded that agglutination tests of sera of geese for the detection of Salmonella carriers is of negligible value since agglutinins may be present for several types of Salmonella. Jungherr and Wilcox (1934) and Gauger *et al.* (1940) tested pigeons for paratyphoid, and Cunningham (1941) used serological procedures in an attempt to control paratyphoid infection in quail.

Various procedures have been recommended for the preparation of antigens for use in conducting serological tests for paratyphoid of poultry (Williams and MacDonald, 1955). The techniques usually employed in the United States for the preparation of tube agglutination antigen closely follow those recommended by McNeil and Hinshaw (1951), the Committee on Salmonellosis of the North Central Regional Poultry Disease Conference (Pomeroy *et al.*, 1957b), or the National Poultry and Turkey Improvement Plans

(Anon., 1963b). Yamamoto *et al.* (1962) also described methods for the preparation and standardization of *S. typhi-murium* antigen for testing turkeys. It is recommended that the O antigen is prepared from nonmotile cultures of the organism and the phase 1 and phase 2 H antigens from properly suppressed, motile cultures. The organisms for O antigen may be grown on beef heart infusion agar and harvested after incubation for 24–48 hours at 37°C. Organisms for the H antigen are cultivated on the above medium or in a liquid broth. The O antigen is harvested in a phenolized saline solution, and the H antigens are treated with formalin. Antigen suspensions are standardized and used in the same manner as pullorum tube agglutination antigen. The phase 1 and phase 2 H antigens are combined to make a single antigen before use. The tests with O antigen are incubated at 37°C. or 50°C. for 24 hours and tests with H antigen at 50°C. for 4 hours or 37°C. for 24 hours before reading. A diagnostic dilution of 1–25 is recommended for routine use. The O antigen is apparently more useful than the H antigen for the detection of carriers on initial tests.

Buxton and Gordon (1947) found that an alcoholized antigen is preferable to a heat-treated broth antigen in the detection of O agglutinins. Gauger *et al.* (1940) and Gwatkin and Grinewitsch (1955b) experimented with whole-blood antigens for the detection of *S. typhi-murium* infection in naturally and artificially infected fowl. Infected birds were not always detected by the antigens used. Clarenburg and Romijn (1954) using *S. bareilly* stained whole-blood antigen, were able to detect several infected birds; however, a large number of nonspecific reactions occurred with the antigen.

Blaxland *et al.* (1958) described the preparation and use of a stained, whole-blood *S. typhi-murium* antigen for testing chickens, turkeys, and ducks by the rapid, macroscopic plate method. The test with this antigen was found to be in close agreement with the tube agglutination test when dealing with birds revealing typically positive reactions. More nonspecific reactions were encountered with the *S. typhi-murium* stained antigen than with conventional *S. pullorum* plate antigens.

Harris and Williams (1957) demonstrated a bacterial hemagglutinin in 17 of 22 strains of *S. typhi-murium* examined. The hemagglutinin was heat labile and disappeared upon prolonged storage of cultures. The results of this study indicated the necessity of carefully selecting strains free of hemagglutinating activity for the production of diagnostic antigens that are to be used with avian whole blood.

Sieburth and Johnson (1956) and Sieburth (1957a, c; 1958b) have described an indirect hemagglutination test that may be of value in screening for the detection of *Salmonella* infections of poultry. Chicken erythrocytes sensitized with material liberated from boiled bacterial cells are used as the antigen in the test. A single polyvalent antigen was found to be capable of detecting several antigenic types of the infection. Hemagglutinins occurred in the blood of infected birds at a higher titer and appeared earlier than conventional agglutinins. It was stated that the indirect hemagglutination test, unlike the agglutination test, detected antibodies in chickens receiving furazolidone treatment. Sieburth (1960) further refined the antigen for the indirect hemagglutination test by developing methods for the production of more stable and standard antigen preparations.

Rice *et al.* (1960) applied the modified direct complement-fixation test for the detection of *Salmonella* antibodies in heat-inactivated turkey serum. Reactions recorded with partially purified somatic antigens were group specific. Magwood and Annau (1961) adsorbed crude and partially purified extracts containing *Salmonella* somatic antigens on polystyrene latex particles. The latex particles were used in agglutination tests to detect *Salmonella* agglutinins in turkey sera. The antigen revealed serological specificity and was agglu-

tinated by antibodies for the homologous Salmonella groups. Results with this testing procedure appeared encouraging, but the practical value of the test in naturally infected birds has not been determined.

In 1965 there was no official testing program for the control of paratyphoid infections on a nationwide basis. Heemstra (1952) pointed out the responsibility of the veterinary profession in helping the

poultry industry seek a solution to this problem. The application of serological procedures for the diagnosis of paratyphoid infections is dependent to a large degree on facilities available in state laboratories for conducting the tests. Furthermore, the importance of the paratyphoid problem in a particular area will indicate the desirability of using testing procedures as an adjunct to the general control program for the disease.

Arizona Infections

The Arizona group consists of motile, lactose-fermenting bacteria that conform to the general definition of the family Enterobacteriaceae. These organisms, also referred to as paracolon bacteria since their recognition in 1939, are closely related to members of the Salmonella and Citrobacter groups, but can be distinguished from them by biochemical tests and serological typing.

Edwards et al. (1959) and Edwards and Ewing (1962) have called attention to the vagueness and taxonomic inaccuracy of the term "paracolon infections" and do not support its further usage in modern bacteriological nomenclature. The term paracolon will be found at places in the following pages of this chapter in deference to its continued use by some and in the interest of accuracy of prior literature citations. As used here, it is not intended to denote a specific group of bacteria.

It was recognized early that there are no sharp limits to this intermediate group of organisms, and any classification that one might formulate must be open to a broad interpretation. Formerly paracolon bacteria have been accepted as occupying a position intermediate between typical coliforms and the paratyphoids, possessing certain characteristics of each. Most of the pathogenic organisms in the group were earlier thought to have the characteristic of fermenting lactose only after prolonged incubation.

The exact distribution, incidence, and

economic importance of Arizona infections have not been determined; however, the growing literature indicates that these infections are being encountered more frequently particularly among young turkeys. Hinshaw and McNeil (1946a), Edwards et al. (1956, 1959), and Goetz (1962) point out that Arizona infections pose a problem of considerable economic importance to the turkey industry.

ETIOLOGY

Stuart et al. (1943) and Borman et al. (1944) classified the paracolon organisms in a specific genus, Paracolobactrum, an appendix to the tribe Eschericheae, and recognized three species, namely Paracolobactrum aerogenoides, Paracolobactrum intermedium, and Paracolobactrum coliforme. This general classification was adopted by the seventh edition of Bergey's Manual of Determinative Bacteriology (Breed et al., 1957) with the addition of the species, Paracolobactrum arizonae.

In a second classification scheme, three main groups of paracolons were recognized with each group being composed of a large number of serologically and biochemically related members. These groups were designated Arizona, Bethesda-Ballerup (Kauffmann and Moeller, 1940; Stuart et al., 1943; Barnes and Cherry, 1946; Edwards et al., 1948d; Moran and Bruner, 1949; West and Edwards, 1954; and Lukas and Bradford, 1954), and Providence (Ewing et al., 1954)

Members of these groups that were pathogenic for poultry, other animals, and man were all included in the Arizona group which is the subject matter of this section. Bethesda-Ballerup and Providence paracolons were not found to be associated with disease outbreaks in poultry. In the discussion that follows, the former Bethesda-Ballerup paracolons are included in the Citrobacter group and the former Providence group is not discussed here as it has been placed with the Proteus group to which it is closely related (Edwards and Ewing, 1962).

Arizona group. In morphology and staining, members of the Arizona group resemble other enteric organisms. They are Gram-negative, nonsporogenic rods which are usually motile by means of peritrichous flagella. Most Arizona strains grow best at 37° C. and are facultative anaerobes with optimum growth under normal atmospheric conditions. The organisms can be readily cultivated on ordinary laboratory media, revealing an abundant growth similar to that of the paratyphoids. Many strains produce a putrid odor, although this characteristic is not consistent.

The first representative of the Arizona group was isolated from fatal infections of certain reptiles by Caldwell and Ryerson (1939). These investigators classified the organism as a *Salmonella* sp. (Dar-es-salaam, var. from Arizona). Kauffmann (1941), after a careful study of the flagellar antigens of one of the original cultures, designated the organism *S. arizona*. It was recognized that the Arizona culture fermented lactose and liquefied gelatin, and Kauffmann (1945) agreed that this organism should be classified separately from the Salmonella.

Investigations by Peluffo *et al.* (1942), Edwards *et al.* (1943, 1947, 1956, 1959), and Edwards and Ewing (1952, 1962) have indicated the biochemical and antigenic similarity, as well as the definite pathogenic properties of strains that make up the Arizona group.

Cultures possessing the following characteristics are almost invariably classifiable serologically as members of the Arizona group (Kauffmann, 1956; Anon., 1958b; Ewing and Edwards, 1960; and Edwards and Ewing, 1962):

Dextrose	— Fermented with gas
Lactose	— Fermented usually promptly
Sucrose	— Not fermented as a rule
Mannitol	— Fermented with gas
Maltose	— Fermented with gas
Dulcitol	— Not fermented
Salicin	— Not fermented
Sorbitol	— Fermented with gas
Adonitol	— Not fermented
Inositol	— Not fermented
Indol	— Not produced as a rule
Methyl Red	— Positive
Voges-Proskauer	— Negative
Simmons' Citrate	— Utilized
H₂S	— Positive
Urea	— Not hydrolyzed
Gelatin	— Liquefied slowly
KCN	— Negative as a rule
Nitrates	— Reduced
Motility	— Positive
Decarboxylases	
Lysine	— Positive
Arginine	— Positive usually delayed
Ornithine	— Positive
Malonate	— Positive
Phenylalanine Deaminase	— Negative

The majority of Arizona cultures ferment lactose promptly but some produce acid from lactose only after 7 to 10 days incubation. The procedures of sealing fermentation tubes with paraffin wax or serially passing the cultures in lactose broth hasten the fermentation of lactose. Edwards *et al.* (1956) reported that cultures of one serotype characteristically failed to ferment lactose. Occasional strains have been encountered which ferment sucrose rapidly, but these are the exception.

The majority of Arizona cultures grow very well on SS agar (Difco) as well as the other solid media recommended for the isolation of Salmonella. On initial isolation on these media, the colonies usually appear clear and colorless like the Salmonella, but may develop a pink or red color after continued incubation for several days or weeks. Rapid lactose-fermenting strains cannot be distinguished from normal coliforms, which are usually inhibited by these media. Mushin (1949) found desoxycholate

agar more effective than SS agar in the isolation of Arizona cultures from humans.

Felsenfeld and Young (1941) reported a medium containing 1 per cent each of lactose and sucrose, and one half of 1 per cent salicin in 0.3 per cent agar for the differentiation of paracolon bacilli and Salmonella. Chilton and MacDonald (1946), Colichon (1952, 1953), Sieburth (1957b), and Edwards and Fife (1961) have also described media for the differentiation of Salmonella and Arizona cultures. Bruner and Peckham (1952) reported the use of Selenite F as a liquid enrichment for the isolation of Arizona from poults.

Arizona strains are often mistaken for Salmonella since they are related so closely both biochemically and serologically; however, such errors are not of extreme importance since the organisms of both groups produce clinically identical diseases in poultry and prevention and control measures are the same for both. The failure of Arizona cultures to ferment dulcitol and their slow liquefaction of gelatin are most useful in distinguishing them from members of the Salmonella group. A summary tabulation of biochemical and other differential tests for identification of paratyphoid, Arizona, and Citrobacter strains is presented in Table 9.3.

Ellis et al. (1957) tested a total of 1,136 Salmonella and 621 Arizona cultures for their ability to utilize the organic acids D-tartrate, citrate, mucate, and malonate during an incubation period of 20 hours. Reaction patterns on these media were divisible into 2 groups, one of which was composed almost exclusively of Salmonella and the other essentially of Arizona strains. Edwards et al (1959) utilized these organic acids in further differential tests for Salmonella and Arizona strains. Especially useful for the purpose is the organic acid sodium malonate which is utilized (+) with Arizona strains and not utilized (−) with Salmonella cultures. Tests should be read after 24 and 48 hours (Edwards and Ewing, 1962).

Citrobacter group. For purposes of classification and identification, the Arizona group of bacteria must be differentiated not only from the Salmonella, but also from a second closely related group, the Citrobacter. Members of this group are not known to be pathogenic for poultry, but from a diagnostic standpoint may be confused with Salmonella and Arizona cultures on initial isolation from fecal specimens. The former Bethesda-Ballerup paracolons (Pc. intermedium) are included in the Citrobacter group along with cultures previously classified as Escherichia freundii. While members of the Citrobacter group are antigenically different from the Salmonella and Arizona groups they must be differentiated from them biochemically.

Members of the Citrobacter group are motile and usually ferment lactose; however, some cultures attack lactose slowly or not at all. Sucrose and salicin may or may not be fermented; indol is usually negative; gelatin is usually not liquefied; and dulcitol is usually fermented.

The potassium cyanide and ninhydrin tests are most valuable in distinguishing Citrobacter strains from those of the Arizona and Salmonella groups. Citrobacter strains grow in KCN medium while Arizona and Salmonella strains, as a rule, do not. Extreme caution must be taken in working with KCN medium to avoid its toxic effects to human beings. Citrobacter strains are ninhydrin negative while Arizona and Salmonella strains are positive. Procedures for conducting both the ninhydrin and KCN tests are described by Edwards and Ewing (1962).

ANTIGENIC STRUCTURE

Antigenically Arizona strains are similar to Salmonella and procedures for the serological study of both groups are identical. Members of the Arizona group have distinct antigenic characteristics that have afforded a means of antigenic analysis and grouping similar to that of the Kauffmann-White schema for Salmonella. Schiff et al. (1941) reported 1 indol-negative paracolon with the complete somatic antigens of S. onderstepoort and 4 others with all or a part of the somatic antigens of other

Salmonella. Edwards et al. (1948) isolated 1 paracolon culture with all the somatic and all but a minor flagellar antigen of a Salmonella type.

Sanders et al. (1945) and Burton and Garrard (1948) reported that atypical reactions observed in routine testing with S. pullorum antigen may be due to certain paracolon strains. The organisms may occasionally be recovered from the tissues of adult chickens and turkeys submitted for routine bacteriological examination as reactors to the blood test for pullorum disease (Gauger, 1946).

By examining the somatic and flagellar antigens of 382 Arizona cultures, Edwards et al. (1947) were able to demonstrate 25 O groups and 61 distinct serological types. Edwards et al. (1956) reported the examination of 1,308 cultures of Arizona among which 96 serotypes were recognized. Edwards and Ewing (1962) recognized 33 O antigen groups and 231 Arizona serotypes. In their diagnostic schema, the above investigators have designated both the somatic and flagellar antigens by Arabic numerals; thus, the original Arizona culture of Caldwell and Ryerson is designated antigenically as Ar. 0–1,2:H–1,2,5, or simply Ar. 1,2:1,2,5. This is the type species of the Arizona group.

Edwards et al. (1956, 1959) found serotype Ar. 7:1,7,8 to be more frequently isolated than any other type in the United States and reported that it comprised almost one-fourth of the total cultures studied and was isolated from 408 outbreaks of infection. In 1947 most of the outbreaks of Ar. 7:1,7,8 infection in poults were from eggs produced by a single cooperative turkey breeder association in a western state. This type is now found in turkeys in all parts of the United States. Moran (1959a) serologically typed 155 members of the Arizona group isolated from animals. A total of 138 of the cultures was isolated from turkeys and was confined to 2 serological types, namely 7:1,2,6 and 7:1,7,8. The number of each of these was approximately equal to the number of cultures of S. typhi-murium typed in 1957 from

turkeys, and combined was nearly twice as great. Arizona strains were not found to occur frequently in chickens. Moran (1960) reported the serological typing in 1958 of 119 Arizona strains, 74.7 per cent of which were isolated from turkeys and only 4.2 per cent from chickens. In 1959 the same author (Moran, 1961a) typed 91 Arizona cultures from chickens and turkeys, of which 93 per cent were isolated from turkeys and 7 per cent from chickens.

HOST DISTRIBUTION AND PATHOGENICITY

Edwards et al. (1947, 1956, 1959) have reported the isolation of Arizona strains from turkeys, chickens, canaries, ducks, parrots, a pheasant, a macaw, reptiles, swine, dogs, a mink, a cat, a Gila monster, a capybara, monkeys, guinea pigs, and opossums. Arizona cultures isolated from fowls and reptiles over a period of 20 years composed almost 75 per cent of the cultures studied. In 1939 when these infections were first recognized in turkeys, poults from individual hatcheries were found to harbor a single serotype of Arizona infection and it was possible to trace the source of the infection to certain hatcheries. Through the interchange of eggs and supply flocks this situation changed and infection with multiple types of Arizona organisms became the rule. The organisms were also recovered from man. Many of the cultures were from normal carriers.

Fey et al. (1957) discussed pathological changes encountered in 6 cases of Arizona infections of reptiles and described the biochemical and serological properties of the causative organisms isolated. Edwards et al. (1961) reported the isolation of 7 new Arizona types from reptiles. Like the paratyphoids, the Arizona organisms apparently recognize no host barriers, and are widely distributed in nature. Goetz and Quortrup (1953) reported the isolation of Arizona strains from gophers, and Hinshaw and McNeil (1947) cited lizards as carriers of the organisms.

Young chicks and poults are most frequently infected during the first 3 weeks

after hatching. Goetz and Quortrup (1953) recorded that the mortality rates among poults infected with Arizona 7:1,7,8 varied from 0.5 to 50 per cent, and occurred from the fourth or fifth day through the third week. Arizona infections in adult birds do not seem to be a problem, although such birds may serve as carriers of the organisms. Like the Salmonella, Arizona bacteria have a tendency to invade the blood stream and the mortality may be high (Edwards et al., 1956). Lewis and Hitchner (1936) reported the isolation of paracolon bacteria from young chicks suffering from an infection which simulated pullorum disease. The mortality rate ranged from 32 to 50 per cent in several broods which had been obtained originally from the same hatchery. These investigators were able to reproduce the disease under laboratory conditions in 1-day-old chicks by subcutaneous and oral inoculations. Guinea pigs were also found susceptible to the infection by parenteral inoculation.

Edwards et al (1947) demonstrated the pathogenicity of an Arizona strain by administering the organisms orally to a group of White Leghorn chicks. Deaths continued for 1 week with a mortality of 16.6 per cent. Of a group of Rhode Island Red chicks which were placed in the same pens as contacts, 26.6 per cent died. Arizona bacilli serologically identical with the organisms administered were recovered from the liver and intestines of every chick that died.

Perek (1957) isolated Pc. aerogenoides from a severe outbreak of paracolon infection in chicks in Israel. The organism proved to be highly infectious for chicks up to 4 weeks of age, but not for adult birds. Bigland and Quon (1958) reported 8 outbreaks of Ar. 7:1,2,6 infection in poults, chicks, and an adult hen in Alberta. This was the first time that Arizona infections had been found among poultry in Alberta. Mortality occurred up to 4 weeks of age and varied from 10 per cent in chicks to 50 per cent in poults.

Peluffo et al. (1942) and Edwards et al.

(1943) reported a group of Arizona strains which were isolated from poults as well as adult turkeys under natural conditions. Mortality varied from 15 to 60 per cent. The average mortality was 35 per cent. Cultures isolated from the same group of birds were of the same serological type and the birds had a common hatchery source.

Hinshaw and McNeil (1944b) reported the occurrence of Arizona type 8 as a cause of mortality in poults. They also reported the presence of this Arizona type in snakes found on the same premises and believed to be carriers of the infection. In further investigations Hinshaw and McNeil (1946a, b) isolated a number of cultures of Arizona serotype 7:1,7,8 from poults and snakes. On one ranch, where a 70 per cent mortality occurred, the greatest losses were observed during the first 5 weeks, but losses continued for 5 weeks. Gauger (1946) isolated Arizona 7:1,7,8 from an adult turkey.

Bruner and Peckham (1932) reported the isolation of Arizona 7:1,7,8 from an outbreak of Arizona infection in poults causing a mortality of 5 per cent. Galton (1953) isolated 13 cultures of Arizona type 10:1,2,5 in a poultry processing plant. Pomeroy et al. (1957a) reported the study of 120 outbreaks of Arizona infections in young turkeys. Four serotypes of Arizona were isolated. Serotype 7:1,7,8 was encountered most frequently. Edwards et al. (1959) found that the number of cultures isolated from turkeys greatly outnumbered those from chickens.

Dougherty (1953) cited two occasions on which he isolated Arizona organisms from duck livers revealing lesions very similar to those produced by paratyphoid infections. Edwards et al. (1947) indicated that Arizona bacteria may cause heavy losses among canaries in which the bacteria may be isolated from ocular tissues in cases of iritis. Edwards et al. (1959) reported that Arizona 13:13,14 was the only causative agent recognized in two highly fatal outbreaks of disease in canaries. Jones et al. (1932) described a type of paracolon encountered in many cases of infectious diar-

rhea in cattle. Johnson et al. (1951) reported infection of the bovine udder with paracolon bacteria. Ryff and Browne (1952) isolated a diphasic Arizona type (26:29,30) from aborted fetuses in ewes.

Reports dealing with the isolation of Arizona strains from man are numerous. Stuart et al. (1943) have indicated that these organisms are often associated with mild or acute gastroenteritis of short duration. Paracolons have also been associated with other pathological conditions in man including chronic enterocolitis (Luippold, 1947), pneumonia (Kraft, 1951), and endocarditis (Friedman and Goldin, 1949). Mushin (1949) found paracolon bacilli to be frequently associated with gastroenteritis of humans in Australia. Lystad (1962) studied 51 strains of Pc. acrogenoides responsible for a nasocomial outbreak of urinary tract infections in man. Evidence indicates that a cycle similar to that which exists between Salmonella infections in man and animals may exist with the Arizona group, and the importance of animals and animal products such as meat and eggs in spreading the infection to man must be considered. Galton (1956) and Edwards et al. (1956, 1959) reviewed the occurrence of cultures of the Arizona group in man.

MODES OF TRANSMISSION

Arizona infections are spread in a manner very similar to paratyphoid infections. Egg transmission of the disease in turkeys has been reported by Edwards et al. (1943), Hinshaw and McNeil (1946a), Edwards et al. (1947), Bruner and Peckham (1952), Goetz et al. (1954), Jamison (1956), and Edwards et al. (1956, 1959). Goetz et al. (1954) were able to isolate Arizona 7:1,7,8 from the eggs and embryos of turkey hens reacting to the agglutination test for this serotype.

Evidence has been presented to indicate that Arizona infection of a particular type may become established in a hatchery and then be spread to a distant establishment through eggs purchased from the original infected hatchery. Edwards et al. (1947) cited instances in which Arizona infection

with organisms of a certain serological type was traced from egg sources in California to points as far away as Minnesota, North Carolina, and Pennsylvania. Bigland and Quon (1958) suspected that Arizona infections were introduced with eggs shipped into Canada from certain western states of the United States. Edwards et al. (1959) in an epizootiological study of Ar. #10:1,2,5 infection in chickens traced the spread of the disease from a southern state to the Midwest and then back to the broiler producing areas of the Southeast. This same type of the organism has appeared in man, dogs, and cats.

Edwards et al. (1943) pointed out that Arizona infection may be carried for long periods by adult fowl which were infected as poults. Sadler et al. (1961) reported the isolation of 2 Arizona strains from intestinal samples of turkeys during a Salmonella survey. Jamison (1956) noted that since this disease usually started in poults in California in late April or May, it was suspected that the breeding flocks were infected from some outside source. Fecal contamination may spread the infection from other animal species to poultry. Goetz (1962) found an Arizona infection rate of 90 per cent in rats and 50 per cent in mice on the premises of a turkey ranch where the infection was a problem in poults. McClure et al. (1957) reported the isolation of several types of paracolon bacteria from the droppings of wild birds in the vicinity of Tokyo, Japan. No Salmonella was isolated from these specimens.

Hinshaw and McNeil (1946a) and Gauger (1946) reported the recovery of the organisms from the ovaries of adult turkeys. The frequent isolation of members of the Arizona group from egg powder by Edwards et al. (1947) suggested the wide distribution of the bacteria in chickens throughout the United States.

Arizona infections are transmitted in the incubator and brooder by direct contact and through contaminated feed and water. The role of contact in spreading the infection among chicks was illustrated by the experiments of Edwards et al.

(1947), already cited. Goetz et al. (1954) did not feel that Arizona infections are frequently transmitted to healthy poults during the brooding period. Erwin (1955) reported the isolation of 73 Paracolobactrum cultures during the bacteriological examination of 206 prepared poultry feed samples.

SYMPTOMS AND LESIONS

The symptoms and necropsy findings in Arizona infections of fowl simulate those of salmonellosis. While symptoms are not specific, infected birds may appear listless, develop diarrhea, and pasting of the down around the vent (Hinshaw and McNeil, 1946a). Bruner and Peckham (1952) reported that infected poults revealed signs of weakness, a tendency to rest on their hocks, ataxia, and trembling. Jamison (1956) reported nervous symptoms in Arizona infections of poults as a result of the organisms localizing in the brain. Perek (1957) observed that death from Arizona infection in chicks was accompanied by convulsions. Bigland and Quon (1958) noted that Arizona infections in chicks and poults revealed sudden deaths preceded for an hour or two by shivering, huddling, and anorexia. In birds 2-3 weeks old, diarrhea, droopiness, closed eyes, twisted heads, and evidence of blindness in one or both eyes were seen up to 2 days prior to death. Infected adults usually reveal no noticeable symptoms.

The lesions described by Lewis and Hitchner (1936) in chicks artificially infected with paracolon bacteria were typical of a generalized septicemia and included peritonitis; retained yolk sacs; enlarged yellowish, mottled or inflamed liver; and discolored heart. Similar lesions were noted in chicks by Edwards et al. (1947), and in poults by Bruner and Peckham (1952). Hinshaw and McNeil (1946a) found marked congestion of the duodenum, and ochre or mottled livers in Arizona-infected poults.

Eye lesions resulting in opacity and partial or complete blindness seem to be quite common in some types of Arizona infections in poults. Jamison (1956) called attention to the cloudiness of the eyes in infected poults which is also observed in Newcastle disease. The organisms can be readily recovered from the infected eye tissues. Bigland and Quon (1958) described eye lesions as a common occurrence in Arizona infections. They found a heavy, yellowish-white, cheesy exudate covering the retinae. Affected eyes became quite desiccated and failed to grow normally. Other lesions included distention of the gallbladder, caseation of the ceca, and tiny lung abscesses. Hinshaw and McNeil (1946a) reported the recovery of Arizona organisms from adult turkeys, two of which had small, caseous mesenteric lesions and three had cystic ovules.

Histological changes due to Arizona infection of chicks were observed by Perek (1957) as fatty degeneration of the liver and distinct capillary congestion of the kidneys.

DIAGNOSIS

Symptoms and lesions are of little value in differentiating Arizona infections from paratyphoid or pullorum disease. Findings on necropsy must be substantiated by recovery and identification of the causative bacteria. In Arizona infections the organisms can usually be recovered from the liver, heart blood, lungs, kidneys, unabsorbed yolk, and intestines. Bigland and Quon (1958) were able to readily isolate Arizona strains from the caseous material covering the retinae of affected eyes. They called attention to the need to differentiate this infection from that due to Aspergillus fumigatus when eye involvement occurs.

Cultural procedures identical to those for paratyphoid infections are employed. Moran (1959b) has reported procedures and media for the isolation and identification of Arizona strains from fresh specimens. The history of the outbreak, as well as the degree of infection indicated by culture results, must be taken into consideration. Recovery of the organisms from the intestinal tract alone may be of little significance. Goetz and Quortrup (1953) used

the cloacal swab and culture technique in an effort to detect carriers of Arizona organisms in adult turkey flocks which had suffered a severe outbreak as poults. Results were entirely negative.

Arizona organisms may be discarded as coliforms, and many probably are. When extended incubation is not practiced, they may be classified as Salmonella strains on initial isolation. The mounting evidence of the pathogenicity for fowl of members of the Arizona group indicates the necessity for studying this group of organisms more closely. Typical reactions of Arizona cultures on selected diagnostic media are listed in Table 9.3. Edwards and Fife (1961) described a lysine-iron agar for the detection of Arizona strains that rapidly ferment lactose. Salmonella and Arizona strains produce a distinctive reaction as they regularly form lysine decarboxylase rapidly and produce large amounts of hydrogen sulfide. Serological analysis of cultures is essential in epizootiological studies of Arizona infections of fowl. If facilities for complete identification of the organisms are not available, cultures should be submitted to the U.S.D.A.'s Diagnostic Services, National Animal Disease Laboratory, Box 70, Ames, Iowa, where the cultures may be studied more closely biochemically and typed antigenically. Goetz et al. (1953) reported the use of a polyvalent antiserum in screening cultures that were suspected to be Arizona strains.

TREATMENT

The use of chemical therapeutic measures may reduce losses in acute outbreaks of Arizona infections, and may be recommended to prevent the spread of the disease in market flocks. Hinshaw and McNeil (1946a) in their study of Ar. 7:1,7,8 infection of turkeys reported the administration of sulfamerazine to a group of 8-day-old poults that had suffered a 12 per cent mortality previous to this treatment. The drug was used at a dosage of 0.25 per cent in the mash for 3 days. Marked improvement was noted in the treated group as compared to untreated

controls. Cessation of treatment resulted in increased losses. A favorable response was noted when the same treatment was administered to both groups of birds for 3 more days. When the survivors were tested with the agglutination test 6 months later, no reactors were found. Goetz et al. (1954) reported that the use of various combinations of sulfonamides and antibiotics had little effect upon mortality from Arizona infections of poults.

Harwood (1956), Jamison (1956), and Pomeroy et al. (1958) have reported on the use of furazolidone (nf-180) in the treatment of Arizona infections. Jamison (1956) described the experimental use of furazolidone in the feed of adult turkey flocks in California as a preventive against Arizona infections. It was recommended that 100 grams of the drug be added to each ton of feed for 1 week each month. The same level of the drug for 5 to 7 days was recommended in treating acute outbreaks of the disease in poults. This level of treatment was also suggested for the first 2 weeks of brooding as a preventive in poults from breeding flocks suspected of having Arizona infections. Higher levels of furazolidone may be recommended and prove more effective in treating particularly severe outbreaks. It is possible that treated birds may remain intestinal carriers of the causative organism. Harwood (1956) has suggested the use of a combination treatment consisting of a sulfonamide in the drinking water and furazolidone in the feed for treatment of severe outbreaks of the disease.

Pomeroy et al. (1958) found that furazolidone fed to poults at a level of 100 grams or higher per ton reduced the mortality from Arizona infection 50 per cent when administered at the time the poults were experimentally exposed to the organisms. The 200 gram per ton level of the drug was found to be more effective in reducing poult mortality and in eliminating Arizona isolations from surviving birds. When the treatment was started before exposure, the 100 gram per ton level of the drug was found to have a preventive effect

on development of the disease. Goetz (1962) reported that intensive furazolidone treatment of laying turkeys infected with Arizona did not eliminate the presence of the organisms in the eggs of such turkeys.

PREVENTION AND CONTROL

Since Arizona infections of fowl are transmitted in the same manner as paratyphoid infections, the control program for this disease is identical to that for paratyphoid infection as outlined earlier in this chapter.

Jamison (1956) found Arizona bacteria more resistant to formaldehyde fumigation than *S. pullorum*. It was recommended that eggs suspected of carrying Arizona organisms should be fumigated with 3 times the amount of formalin-potassium permanganate mixture used to fumigate for pullorum disease. Fumigation should start as soon as the eggs are set and the temperature and humidity are up to regular operating levels. Eggs should never be fumigated between the 24th and 96th hours of incubation. It was recommended that the eggs be fumigated again with the triple strength fumigant just before they are transferred to the hatching trays. If continuous fumigation is used, the regular level of the fumigant was suggested.

Because of the large number of antigenic types of Arizona strains, any control pro-

gram involving the use of the agglutination test to detect infected adults must be based on the location and disposal of infected breeding flocks. Specific types of antigen must be prepared and used in each case. From the small percentage of reactors to the agglutination test found by Hinshaw and McNeil (1946a), it would appear that the infection may be more easily shed than is pullorum disease. Goetz *et al.* (1954) used an experimental plate agglutination antigen to test serum samples from turkey flocks infected with Arizona 7:1,7,8. In one flock containing 1,414 birds, 6 reactors were found, 5 of which yielded Arizona on culture. A retest of the flock 1 month later revealed no reactors. Other flocks tested contained many reactors and were disposed of. Goetz (1962) described the use of a flagellar tube agglutination test for Arizona infection in California turkeys. Reference may be made to the section on Prevention and Control of paratyphoid infections for a discussion of the California program for the control of Arizona infections in turkeys.

The establishment of the Arizona as a group of considerable pathogenic importance for fowl suggests that all efforts aimed at the prevention and control of paratyphoid infections must also take into consideration these closely related organisms.

REFERENCES

Abelseth, M. K., and Robertson, H. E: 1955. *Salmonella typhimurium* infection in 1952 turkey flocks—A public health hazard. Canad. Jour. Pub. Health 44:263.

Adler, H. E., Nilson, M. A., and Stadelman, W. J.: 1953. A study of turkeys artificially infected with *Salmonella typhimurium*. Am. Jour. Vet. Res. 14:246.

———, Willers, E. H., and Levine, M: 1951. Incidence of *Salmonella* in apparently healthy dogs. Jour. Am. Vet. Med. Assn 118:300

Akiyama, T.: 1961. Studies on *Salmonella* infections in chicks. II. Observation on chicks hatched from eggs artificially infected with *S. pullorum* and *S. senftenberg*. Japanese Jour. of Bact. 16.460.

———, Watanabe, S., and Sakaraki, R.: 1959. A survey on *Salmonella* infection among chicks and embryonating eggs in Aomori Prefecture. Jour. Japanese Vet. Med. Assn. 12:210.

Altman, I. E.: 1940. *Salmonella suspecster* infection in canaries. Jour. Am. Vet. Med. Assn. 97.601.

Alves de Oliveira, J. J., and Gomes, J F· 1954 Isolamento e caracterização de *S. typhimurium* em ovos de galinha, por ocasião de um surto ocorrido no hospital da C.U.F. Rev. Ciênc. Vet. 49.389.

Anderson, A. S., Bauer, H., and Nelson, C. B.: 1955. Salmonellosis due to *Salmonella typhimurium* with Easter chicks as likely source. Jour. Am. Med. Assn. 158:1155.

Anderson, E. S., and Wilson, E. M. J.: 1961. Die Bedeutung der *Salmonella typhimurium*-Phagen-Typisierung in der Human- und Veterinärmedizin. Zentralbl. f. Bakt. I orig. 181:568.

Anellis, A., Lubas, J., and Rayman, M. M.: 1951. Heat resistance in liquid eggs of some strains of the genus Salmonella. Food Res. 19 377.

Angstrom, C. I.: 1957. Case report—A paratyphoid outbreak in a poultry breeding flock. Avian Dis. 1:52.

Anonymous: 1947. Outline of a sanitation program for the poultry industry. U.S.D.A., ARS, ADE Div., Washington, D.C.

———: 1954. Purity tests for vaccines of egg-embryo origin. Procedures for the isolation and identification of Salmonella. Biol. Products Memo. 54–1. U.S.D.A., ARS, AIQ, Div., Washington, D.C.

———: 1956. Report public health laboratory service. Food poisoning in England and Wales, 1955. Month. Bul. Med. Res. Counc. (Gt. Brit.) 15:265.

———: 1958a. Report of committee on salmonellosis and related enteric diseases. Rep of Nat. Plans Conf., U.S.D.A., ARS, AHR Division, Beltsville, Md., p 36.

———: 1958b. Report of the Enterobacteriaceae subcommittee of the nomenclature committee of the International Association of Microbiological Societies. Internat. Bul. on Bact. Nomen. and Taxonomy 8.25.

———: 1959. Report, Salmonella organisms in animal feeding stuffs and fertilizers. Month. Bul. Minist. Health Lab. Serv. 18.26.

———: 1961. Infection from poultry. Lancet, January 7, p. 38.

———: 1962. Recommended procedure for the isolation of Salmonella organisms from animal feeds and meat byproducts. ARS 91–56, U.S.D.A., ARS, ADE Division, Washington, D.C.

———: 1963a. Salmonella derby epidemic—Follow-up report. Morbidity and Mortality Weekly Report, U.S. Dept. of Health, Education and Welfare 12:250.

———: 1963b. National poultry and turkey improvement plans and auxiliary provisions. Misc. Publ. 739, U.S.D.A., ARS, AHR Division, Beltsville, Md.

Atkinson, N.: 1956. The occurrence of Salmonella types in Australia. 11. Types found among 3,540 strains. Australian Jour. Exper. Biol. and Med. Sci. 31:569.

Bahr, L., and Christensen, N. P. C.: 1933. Investigations concerning the B. pullorum and bacteria pertaining to the Salmonella group. Brit. Vet. Jour. 89.361.

Ballantyne, E. E.: 1953. Salmonella infections of poultry in Alberta. Proc. Book Am. Vet. Med. Assn. 90th Ann. Meet. p. 355.

Banwart, G. J., and Ayres, J. C.: 1957. The effect of pH on the growth of Salmonella and functional properties of liquid egg white. Food Technol. 11 214.

Barckhausen, J.: 1961. Aufenthalt und Verhalten einer Jungen Blässgans mit Mäusetyphus (Salmonella typhi-murium). Deutsch. tierärztl. Wochenschr. 68:660.

Barnes, L. A., and Cherry, W. B.: 1946. A group of paracolon organisms having apparent pathogenicity. Am. Jour. Pub. Health 46:481.

Beach, J. R.: 1936. Remarks made during discussion of paratyphoid infections of poultry. Proc. 6th World's Poultry Cong. 3:410.

Beattie, W. E.: 1960. Avian infection with Salmonella thompson—Observations. Poultry Sci. 39.1235.

Beaudette, F. R.: 1926a. B. aertrycke infection in canary birds and parrots. Jour. Am. Vet. Med. Assn. 68:642.

———: 1926b. B. aertrycke as the etiological agent in a disease affecting squabs. Jour. Am. Vet. Med. Assn. 68.644.

———, and Edwards, P. R.: 1926. The etiology of a canary bird epizootic. Jour. Bact 12 51.

Becker, W.: 1957. Salmonellosen beim Hausgeflügel. Berl. und Munchener tierärztl. Wochenschr. 70:168.

Béguin, S., and Grabar, J: 1953. Etudes sur les formes rugueuses des Salmonella. Ann. Inst. Pasteur 84.723.

Belding, R. C., and Mayer, M. L.: 1958. Furazolidone in the treatment of Salmonella infections of turkeys. 2. Effect on acute paratyphoid infection in poults. Poultry Sci. 37:463.

Belohn, A., and Schlosser, G. C.: 1963. Adequacy of cooking procedures for the destruction of Salmonellae. Amer. Jour. Pub. Health 53:782.

Bergsma, C.: 1959. Veterinaire Problemen bij de Epidemiologie der Salmonellosen. Tijdschr. Diergeneesk. 84:872.

Buddle, E. S., and Cover, M. S.: 1957. The bacterial flora of the respiratory tract of chickens affected with chronic respiratory disease. Amer. Jour. Vet. Res. 18:405.

Bieter, R. W.: 1960. Effect of age factor on mortality in Salmonella typhimurium infection in turkey poults. Jour. Am. Vet. Med. Assn. 137.657.

———: 1961. A method of inducing Salmonella typhimurium infection in chicks Jour. Am. Vet. Med. Assn. 139:790.

———: 1963. The use of nihydrazone against Salmonella typhimurium and Salmonella gallinarum infections in turkeys. Poultry Sci. 42:465.

———, and Barnett, B. D.: 1964. Effect of increasing wash water temperature on eggs contaminated with Salmonella. Poultry Sci. 40:1379.

———, and Barnett, B. D.: 1962a. Nihydrazone and the Salmonella infections Poultry Sci. 41-1291.

Bierer, B. W., and Barnett, B. D.: 1962b. Furaltadone water medication and the salmonelloses Proc. 12th World's Poultry Cong. p. 283.

———, Barnett, B. D., and Valentine, H. D.: 1961a. Experimentally killing *Salmonella typhimurium* on egg shells by washing. Poultry Sci. 40:1009.

———, Valentine, H. D., Barnett, B. D., and Rhodes, W. H.: 1961b. Germicidal efficiency of egg washing compounds on eggs artificially contaminated with *Salmonella typhimurium*. Poultry Sci. 40:148.

———, Valentine, H. D., and Vickers, D. J.: 1961c. Furaltadone water medication: Its use in avian salmonellosis. Avian Dis. 5:214.

———, and Vickers, D. J.: 1960a. Nitrofuran medication for experimental *Salmonella typhimurium* infection in poults. Avian Dis. 4:22.

———, and Vickers, D. J.: 1960b. Evaluation of water soluble nitrofurans in experimental Salmonella infection in turkey poults. Vet. Med. 55(11):78.

Bigland, C. H., and Papas, G.: 1953. Experiment in egg penetration by Salmonella. Canad. Jour. Comp. Med. and Vet. Sci. 17:105.

———, and Quon, A. A.: 1958. Infections of poultry with Arizona paracolon in Alberta. Canad. Jour. Comp. Med. and Vet Sci. 22:308.

———, Wilton, G. S., Vance, H. N., and Carlson, H. C.: 1962. Salmonellosis of animals in Alberta, 1949-1960. Jour. Am. Vet. Med. Assn. 140:251.

Bischoff, J.: 1960. Über die Weltweite Verbreitung der Keime aus der Salmonellagruppe. Berl. und Munchener tierärztl. Wochenschr. 73:233.

——— 1961. Vorschläge zur Änderung der Verordnung zum Schutze gegen Infektion durch Erreger der Salmonellagruppe in Eiprodukten vom 17. Dezember 1956. Berl. und Munchener tierärztl. Wochenschr. 74:70.

Blaxland, J. D., and Blowers, A. J.: 1951. *Salmonella typhimurium* infection in duck eggs as a cause of human food poisoning. Vet. Record 63:56.

———, Sojka, W. J., and Smither, A. M.: 1958. Avian salmonellosis in England and Wales 1948-56, with comment on its prevention and control. Vet. Record 70:374.

Bliznakow, E. G., Ranson, J. P., and Heller, J. H.: 1963. Modification of *Salmonella typhimurium* infection of embryonated eggs by antiserum, adult splenic tissue and combinations of these agents Proc. Soc. for Exper. Biol. and Med. 112:567.

Bloom, H. H., Mack, W N, and Mallmann, W. L.: 1958 Enteric viruses and Salmonellae isolation. II. Media comparison for Salmonellae. Sewage and Industr. Waste 30:1455.

Borman, E. K., Stuart, C. A., and Wheeler, K. M.: 1944. Taxonomy of the family Enterobacteriaceae. Jour. Bact. 48 351.

Bovre, K., and Sandbu, P.: 1959. Salmonella excreting tortoises in Oslo. Acta Path. et Microbiol Scand. 46 339.

Boyer, C. I., Bruner, D. W., and Brown, J. A.: 1958. Salmonella organisms isolated from poultry feed. Proc. 30th N. E Conf on Avian Dis.

———, Narotsky, S., Bruner, D. W., and Brown, J A.: 1962. Salmonellosis in turkeys and chickens associated with contaminated feed. Avian Dis 6:43.

Brandly, C. A.: 1951 Encephalitis as public health problems. Pub. Health Rep. 66.668.

Breed, R. S., Murray, E. G. D., and Smith, N. R.: 1957 Bergey's Manual of Determinative Bacteriology, 7th ed., Williams and Wilkins Co., Baltimore.

Brest Nielsen, B.: 1960. *Salmonella typhimurium* carriers in seagulls and mallards as a possible source of infection to domestic animals. Nordisk Veterinærmed. 12.417.

Brobst, D., Greenberg, J., and Gezon, H. M.: 1958. Salmonellosis in poultry and poultry processing plants in Western Pennsylvania. Jour. Am Vet. Med. Assn. 133:435.

Browne, A. S: 1949. The public health significance of Salmonella on poultry and poultry products. Doctoral thesis, University of California, Berkeley.

Bruner, D. W 1957 The preparation and use of a polyvalent Salmonella antiserum Cornell Vet. 47:491.

——— and Edwards, P. R: 1941. Microorganisms of the genus Salmonella with special reference to a new Salmonella type: Am. Jour. Hyg. 34 (Sec. B): 82.

——— and Moran, A. B: 1949. Salmonella infections of domestic animals. Cornell Vet. 39.53.

——— and Peckham, M. C.: 1952. An outbreak of paracolon infection in turkey poults. Cornell Vet. 42.22

Burkhart, D. M., Wolfgang, R. W., and Harwood, P. D.: 1962. Salmonellosis in parakeets and canaries treated with nitrofurans in the drinking water. Avian Dis. 6.275.

Burr, W. E., and Helmboldt, C. F.: 1962. Salmonella species contaminants in three animal by products Avian Dis. 6 441.

———, Tourtellotte, M., Lugınbuhl, R E., and Jungherr, E. L: 1957. *Salmonella heidelberg* infection as a problem in pullorum disease control. Avian Dis 1-298.

Burton, W. H., and Garrard, E. H: 1948. Non-pullorum agglutination reactions. IV. Reactions with pullorum antigen from fowl inoculated with coliform types. Canad. Jour. Comp. Med. and Vet. Sci. 12 90.

Butler, R. L., and Mickel, C.E: 1955. Insect and rodent contamination of grain. Minn Agr. Exper. Sta. Bull. 431.

Buxton, A.: 1957a. Salmonellosis in Animals. A Review. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England.

———: 1957b. Public health aspects of salmonellosis in animals. Vet. Record 69:105.

———: 1958. Salmonellosis in animals. Vet. Record 70.1044.

——— and Gordon, R. F.: 1947. The epidemiology and control of Salmonella thompson infection of fowls. Jour. Hyg. Cambridge 45:265.

Caldwell, M. E., and Ryerson, D. L.: 1939. Salmonellosis in certain reptiles. Jour. Infect. Dis. 65:242.

Camle-Parola, E., and Ordal, Z. J.: 1957. A survey of the bacteriological quality of frozen poultry pies. Food Technol. 11:578.

Cantor, A., and McFarlane, V. H.: 1948. Salmonella organisms on and in chicken eggs. Poultry Sci. 27:350.

Chang, T. S., Rheins, M. S., and Winter, A. R.: 1957. The significance of the Bursa of Fabricius in antibody production in chickens. I. Age of chickens. Poultry Sci. 36:735.

Chaplin, W. C., and Hamilton, C. M.: 1957. A synovitis in turkeys produced by Salmonella thompson. Poultry Sci. 36:1380.

Chase, F. E.: 1947. The occurrence and distribution of Salmonella types in fowl. II. Studies of artificial S. bareilly and S. oranienburg infections in hens Canad. Jour. Res. 25 316.

Cherrington, V. A., Gildow, E. M., and Moore, P.: 1937. Paratyphoid in turkeys. Poultry Sci 16:226.

Cherry, W. B., Barnes, L. A., and Edwards, P. R.: 1946. Observations on a monophasic Salmonella variant. Jour. Bact. 51:335.

Chilton, M. L., and MacDonald, F.: 1949. A presumptive medium for differentiating paracolon from Salmonella cultures. Jour. Lab. and Clin. Med. 31:824.

Christensen, W. B.: 1946. Urea decomposition as a means of differentiating Proteus and para-colon cultures from each other and from Salmonella and Shigella types. Jour. Bact. 52:461.

Clarenburg, A.: 1939. Paratyphoid in ducks in relation to public health. Proc. 7th World's Poultry Cong. P. 233.

———, and Roepke, W. J.: 1952. S bareilly—Infectie bij kuikens. Tijdschr Diergeneesk. 77:174.

———, and Romijn, C.: 1954. The effectiveness of fumigation with the formaldehyde-potassium permanganate and the influence on the hatchability. Proc. 10th World's Poultry Cong. P. 214.

Clark, C. H.: 1946. Sulfamerazine in paratyphoid disease of poults and chicks. Jour. Am. Vet. Med. Assn. 109:279.

Clemmer, D. I., Hickey, J. L. S., Bridges, J. F., Schliessmann, D. J., and Shaffer, M. F.: 1960. Bacteriologic studies of experimental air-borne salmonellosis in chicks. Jour. Infect. Dis. 106:197.

Colichon, H.: 1952. New media for the differentiation of enteric bacteria. Pub. Health Rep. 67:401.

———: 1955. Differential mediums for enteric bacteria. Amer. Jour. Clin. Path. 25:506.

Cope, E. J., Appelhof, W. K., and Marcineau, P. C.: 1955. Salmonella isolated from animals, birds, and reptiles in a metropolitan zoo. Cornell Vet. 45:3.

Craige, J. E.: 1944. The isolation of Salmonella anatum from the feces of a dog. Jour. Am. Vet. Med. Assn. 105:33.

Csázár, V., Dózsa, I., and Takács, J.: 1961. A budapesti Állatkertí állatok Salmonellás fertözöttsége. Magyar Álla. Vosok Lapja 16:373.

Cunningham, O. C.: 1941. Paratyphoid infection in quail. Jour. Am. Vet. Med. Assn. 99·217.

Daigeler, A.: 1957. Über das Vorkommen von Salmonellen bei Huhnern. Berl. und Münchener tierarztl. Wochenschr. 70.305.

Dalling, T., and Warrack, G. K.: 1932. Ducks and Salmonella infection. Jour. Path. and Bact. 35.655.

Darby, C. W., and Stafseth, H. J.: 1942. Salmonella infections common to man, animals, and birds. Proc. 46th Ann. Meet. U.S. Livestock Sanit. Assn. P. 189.

Das, M. S., Chakravorty, M. B., and Ghosh, G. K.: 1959. Occurrence of avian salmonellosis in West Bengal, Indian Vet. Jour. 36:403.

Delay, P. D., Jackson, T. W., Jones, E. E., and Stover, D. E.: 1954. A testing service for the control of Salmonella typhimurium infection in turkeys. Am. Jour. Vet. Res. 15:122.

Deom, J.: 1960. Un type rare de Salmonella isolé chez un passereau au Congo Belge. Rev. Path. Gén. 60:219.

Dixon, J. M. S.: 1961. Rapid isolation of Salmonellae from faeces. Jour. Clin. Path. 14.397.

———, and Pooley, F. E.: 1961. Salmonellae in a poultry processing plant. Monlh. Bul. Minist. Health Lab. Serv. 20:30.

Dolman, C. E.: 1954. Some ways in which animal health affects human health. Canad. Jour. Comp. Med. and Vet. Sci. 18:35.

Dougherty, E.: 1953. Disease problems confronting the duck industry. Proc. Book Am. Vet. Med. Assn. 90th Ann. Meet. P. 359.

Dougherty, E.: 1954. Paratyphoid infection in the White Pekin duck. Proc. 26th Ann. Conf. Lab. Workers in Pullorum Disease Control

——: 1961. The pathology of paratyphoid infection in the White Pekin duck, particularly the lesions in the central nervous system. Avian Dis. 5:415.

Doyle, T. M.: 1927. *B. aertrycke* infection of chicks. Jour. Comp. Path. and Therap. 40:71.

Dózsa, L.: 1961. A hazivéréb (*Passer d. domesticus*), mint *Salmonella typhimurium* — Reservoir. Mag. Allator. Lapja 16.144.

Edwards, P. R.: 1929. A fatal infection of chicks due to bacilli of the paratyphoid B group. Jour. Infect. Dis. 43:191.

——: 1935 A serological variant of *S. aertrycke* isolated from pigeons. Jour. Bact. 30:465.

——: 1939. Incidence of Salmonella types in fowls in the United States. Proc. 7th World's Poultry Cong. P. 271

——: 1958. Salmonellosis: Observations on incidence and control. Ann. New York Acad. of Sciences 70:598.

——, and Bruner, D. W.: 1940. The occurrence of multiple types of paratyphoid bacilli in infections of fowls, with special reference to two new Salmonella species. Jour. Infect. Dis 66.218

——, and Bruner, D. W.: 1943. The occurrence and distribution of Salmonella types in the United States. Jour. Infect. Dis. 72:58.

——, Bruner, D. W., and Moran, A. B.: 1948a. The genus Salmonella: Its occurrence and distribution in the United States. Ky. Agr. Exper. Sta. Bul. 525.

——, Bruner, D. W., and Moran, A. B.: 1948b. Further studies on the occurrence and distribution of Salmonella types in the United States Jour. Infect. Dis, 83.220.

——, Bruner, D. W., and Moran, A. B: 1948c. Salmonella infections of fowls. Cornell Vet. 38 247.

——, Cherry, W. B., and Bruner, D. W.: 1943. Further studies on coliform bacteria serologically related to the genus Salmonella. Jour. Infect. Dis. 73 229.

——, and Ewing, W H · 1952. The status of serologic typing in the family Enterobacteriaceae. Am. Jour. Pub. Health 42.655

——, and Ewing, W. H· 1962 *Identification of Enterobacteriaceae.* Burgess Publ. Co. Minneapolis

——, and Fife, M. M. A· 1961 Lysine-iron agar in the detection of Arizona cultures. Applied Microbiology 9:478.

——, Fife, M A., and Ramsey, C H.: 1959. Studies on the Arizona group of Enterobacteriaceae. Bact. Rev 23 155.

——, Kampelmacher, E. H, Fife, M A, and Guinee, P, A.: 1961. Seven new Arizona serotypes isolated from reptiles Antonie Leeuwenhoeck 27:110.

——, McWhorter, A. C, and Fife, M A: 1956. The Arizona group of Enterobacteriaceae in animals and man. Occurrence and distribution. Bul. Organization mond. Santé, Bul. World Health Organization 14 511

——, Moran, A. B., and Bruner, D. W: 1946. Flagella and flagellar antigens in "nonmotile" salmonella cultures. Proc. Soc. Exper. Biol., N.Y. 62:296.

——, West, M. G., and Bruner, D. W.: 1947. Arizona group of paracolon bacteria. Ky. Agr. Exper. Sta. Bul. 499.

——, West, M. G, and Bruner, D. W: 1948d. Antigenic studies of a group of paracolon bacteria (Bethesda group). Jour. Bact. 55:711.

Ellemann, G.: 1959. Temperaturens betydning for indvaekst af *Salmonella typhi–murium* i homesang Nordisk Veterinaermed 11.341.

——: 1960. Undersøgelse af hons og kyllinger 24 et fjerkraeslagteri med henblik på fund af Salmonella-bakterier fra kloaken. Nordisk Veterinaermed. 12.47.

Ellis, C. C.: 1957. Evaluation of current programs of testing turkeys for *Salmonella typhimurium* in Wisconsin Proc. 8th Ann. No. Central Reg. Poultry Dis. Conf.

Ellis, R. J., Edwards, P. R., and Fife, M A: 1957. The differentiation of the Salmonella and Arizona groups by utilization of organic acids Pub. Health Lab. Rep. 15:49.

Emmel, M. W.: 1936a. The etiology of fowl paralysis leukema and allied conditions in animals. III. The intestinal flora of chickens affected with enteritis associated with intestinal parasitism. Florida Agr. Exper. Sta. Bul. 293.

——: 1936b. The importance of endotoxin of *Salmonella aertrycke* in the development of fowl paralysis. Vet. Med. 31:456.

——, and Statseth, H. J: 1929. *Salmonella aertrycke* infection in the canary bird. Jour. Am. Vet. Med. Assn. 75.230.

Erwin, L. E: 1955. Examination of prepared poultry feeds for the presence of Salmonella and other enteric organisms. Poultry Sci. 34:215.

Evans, W. M., Bruner, D. W, and Peckham, M. C: 1955. Blindness in chicks associated with salmonellosis. Cornell Vet. 45,239.

Eveleth, D. F., Goldsby, A. S., and Bohn, F. M.: 1947. The treatment of pullorum disease and paratyphoid infections with sulfamerazine. No Dak. Agr. Exper. Sta. Bimo. Bul. 9:163.

Ewing, W. H., Davis, B. R., and Edwards, P. R · 1960. The decarboxylase reactions of Enterobacteriaceae and their value in taxonomy. Pub. Health Lab Rep. 18:77

———, and Edwards, P. R.: 1960. The principal divisions and groups of Enterobacteriaceae and their differentiation. Internat. Bull. on Bact. Nomen. and Taxonomy 10:1.

———, Tanner, K. E., and Dennard, D. A.: 1954. The Providence group: An intermediate group of enteric bacteria. Jour. Infect. Dis. 94:134.

Feils, C.: 1957. Seltene Salmonella-Typen beim Geflugel unter Berucksichtigung der Pullorum-untersuchungen. Berl. und Munchener tierärztl Wochenschr. 70:308.

Felix, A, and Pitt, R. M.: 1935. Virulence and immunogenic activities of B. typhosus in relation to antigenic constituents. Jour. Hyg., Cambridge 35 428.

Felsenfeld, O.: 1949. Notes on Salmonella cubana. Poultry Sci. 28:142.

———, and Young, V. M.: 1944. Medium for the differentiation of Salmonella and paracolon organisms. Am. Jour. Clin. Path. 14:26.

———, and Young, V. M.: 1945. The viability of Salmonella on artificially contaminated vegetables. Poultry Sci. 24:355.

———, Young, V. M., and Niven, J.: 1950. A survey of Salmonella organisms in market meat, eggs, and milk. Jour, Am. Vet. Med. Assn. 116 17.

Fenstermacher, R.: 1952. Paratyphoid infections. In Diseases of Poultry, 3rd ed. Iowa State College Press, Ames, Iowa P. 288.

Fey, H., and Wiesmann, E.: 1960. Die Gefahr des Salmonellenimportes mit Eiprodukten und Tierischen Futtermitteln. Schweiz. Med, Wochenschr. 90 791.

———, Edwards, P. R., and Stunzi, H 1957 Arizona-Infektionen bei Reptilien mit Isolierung von 4 Neuen Arizonatypen. Schweiz. Zietschr. allg. Path. und Bakt. 20 27

Florin, S. O., and Nilsson, T.: 1959. The influence of slaughter technique on contamination of the meat in cases of intestinal salmonellosis of poultry. Proc. 8th Nordic Vet. Cong., Helsinki, p. 773.

Francis, D. W., Campbell, H., and Newton, G. R.: 1960 The use of furazolidone for chukar partridges. Avian Dis. 4.218.

Frank, J. F., and Wright, G, W.: 1955. Susceptibility of Salmonella organisms to formaldehyde fumigation. Canad. Jour Comp. Med. and Vet. Sci. 19:71.

———, and Wright, G. W.: 1956. The disinfection of eggs contaminated with Salmonella typhimurium. Canad. Jour, Comp. Med. and Vet Sci. 20 406.

Friedman, I. A., and Goldin, M.: 1949. Paracolon endocarditis. Am. Jour. Clin. Path. 19 840.

Galbraith, N. S., Taylor, C. E. D., Cavanagh, P., Hagan, J. G, and Patton, J. L.: 1962. Pet foods and garden fertilizers as sources of human salmonellosis. Lancet, February 17, p. 372.

Galton, M. M.: 1953. Sanitation problems in poultry processing plants. Proc. Pub. Health Vet Meet, Atlanta, Ga., June 15–19, 1953. P. 74.

———. 1956. Poultry diseases transmissible to man including summary report of outbreaks. Cong. Rec., June 18, 1956. P. 9182.

———. 1961. Laboratory procedures for the isolation of Salmonella from human and animal food products. Proc. 60th Ann. Meet. U S Livestock Sanit. Assn. P. 434.

———, and Arnstein, P.: 1960. Poultry diseases in public health: review for epidemiologists. U.S. Pub. Health Serv. Publication No. 767

———, Mackel, D. C., Lewis, A. L., Haire, W C., and Hardy, A. V.: 1955. Salmonellosis in poultry and poultry processing plants in Florida. Am. Jour. Vet. Res. 16:132.

———, Scatterday, J. E., and Hardy, A. V.: 1952. Salmonellosis in dogs. I. Bacteriological, epidemiological, and clinical considerations. Jour. Infect. Dis. 91:1.

Garside, J. S., and Gordon, R. F.: 1940. Salmonella infections of ducks and ducklings. Jour. Comp. Path. and Therap. 53.80.

———, Gordon, R. F., and Tucker, J. F.: 1960. The emergence of resistant strains of Salmonella typhimurium in the tissues and alimentary tracts of chickens following the feeding of an antibiotic. Research in Vet. Sci. 1:164.

Gauger, H. C.: 1946. Isolation of type 10 paracolon bacillus from an adult turkey. Poultry Sci. 25:299.

———, and Greaves, R. E.: 1946a. Bacterial examination of shells and contents of eggs laid by turkeys naturally and artificially infected with Salmonella typhimurium. Poultry Sci. 25:119.

———, and Greaves, R. E.: 1946b. Isolation of Salmonella typhimurium from drinking water in an infected environment. Poultry Sci. 25:476

———, and Greaves, R. E.: 1946c. Isolation of Salmonella typhimurium from the feces of turkeys. Poultry Sci. 25:232

———, and Greaves, R. E.: 1947. Isolation of Salmonella typhimurium from the feces of artificially infected poults. Poultry Sci. 26.48.

———, Greaves, R. E., and Cook, F. W.: 1940. Paratyphoid of pigeons. I. Serological, bacteriological, and hematological studies of spontaneously infected birds. N.C. Agr. Exper Sta. Tech Bul 62

Gaugusch, Z.: 1958. Badania bakteriologiczne jaj kaczych zakazonych naturalnie i sztucznie paleczka S. typhimurium. Med. Weter. 14:393.

———, and Malwińska, I.: 1956. Badania bakteriologiczne naturalnych i sztucznych srodowisk wodnych, przy Salmonelozie ptactwa wodnego. Med. Vet., Warszie 12-276.

Gibbons, N. E., and Moore, R. L.: 1946. A note on artificially infected fowl as carriers of Salmonella. Poultry Sci. 25:115.

Giovanelli, N. E., and Dominguez, O. R.: 1960. Salmonellosis en canarios. Gac. Vet. B. Aires 22:183.

Goetz, M. E.: 1962. The control of paracolon and paratyphoid infections in turkey poults. Avian Dis. 6:93.

——— and Quortrup, E. R.: 1953. Some observations on the problems of Arizona paracolon infections of poults. Vet. Med. 48:59.

——— Quortrup, E. R., and Dunsing, J. E.: 1954. Investigations of Arizona infections in poults. Jour. Am. Vet. Med. Assn. 124.120.

Gordon, R. F.: 1959. Brooder diseases. Vet. Record 71 994.

——— and Buxton, A.: 1915. The isolation of Salmonella thompson from outbreaks of disease in chicks. Jour. Hyg., Cambridge 44:179.

——— and Buxton, A.: 1916. A survey of avian salmonellosis in Great Britain. Brit. Vet. Jour. 102:187.

——— and Garside, J. S.: 1944. Salmonella infections in ducks. Observations on the value of the agglutination test in the eradication of infection and investigations on the cycle of infection via the egg. Jour. Comp. Path. and Therap. 51:65.

——— and Tucker, J. F.: 1957. The isolation of Salmonella infantis from a turkey poult. Month. Bul. Minist. Health Lab. Serv. 16:71.

Graham, R.: 1936. Salmonella isolated from baby quail. Jour. Am. Vet. Med. Assn. 88.765.

——— and Michael, V.: 1936. Studies on incubator hygiene. V. Poultry Sci. 15:85.

Graham-Jones, O., and Fiennes, R. N.: 1959. Dried egg in birds' food. Vet. Record 71:245.

Greenberg, B.: 1959. Persistence of bacteria in developmental stages of housefly. I. Survival of enteric pathogens in normal and aseptically reared host. Amer. Jour. Trop. Med. and Hyg. 8 405.

Gregory, D. W.: 1948. Salmonella infections of turkey eggs. Poultry Sci. 27:559.

Grumbles, L. C., and Flowers, A. I.: 1961. Epidemiology of paratyphoid infections in turkeys. Species encountered and possible sources of infection. Jour. Am. Vet. Med. Assn. 138.261.

Gunnarson, M. F., McFadden, H. W., and Kyle, T. S.: 1954. The Bacteriology of Commercial Poultry Processing. Burgess Publ. Co., Minneapolis.

Gwatkin, R., and Dzenis, L.: 1954. Salmonellosis. I. Agglutination tests in experimental infections in chickens. Canad. Jour. Comp. Med. and Vet. Sci. 18:155.

——— and Grinewitsch, C.: 1955a. Salmonellosis. II. Comparison of whole blood agglutination test and faecal cultures in chickens and turkeys infected by mouth with Salmonella typhi-murium. Canad. Jour. Comp. Med. and Vet. Sci. 19.113.

——— and Grinewitsch, C.: 1955b. Salmonellosis. III. Blood cultures and agglutination tests on chickens infected by mouth with Salmonella typhi-murium. Canad. Jour. Comp. Med. and Vet. Sci. 19:174.

——— and Mitchell, C. A.: 1944. Transmission of Salmonella pullorum by flies. Canad. Jour. Pub. Health. 35 281.

Hamada, S., Hashimoto, H., Tazaka, T., and Tsuchiya, Y.: 1958. Studies on chick salmonellosis. II. Salmonella senftenberg infection in chicks. Japanese Jour. Vet. Sci.

Hammer, B.: 1961. Die Einwirkung der Salmonellose bei Hausratten in Baden unter Berücksichtigung gesicherter Infektionen. Berl. und Münchener tierärztl. Wochenschr. 74 63.

Hansen, A. C.: 1942. Der item Hausgeflügel in Dänemark festgestellten Salmonellatypen. Zentralbl. f. Bakt. I. 149.222.

Harris, M. E., and Williams, J. E.: 1957. The hemagglutinating properties of Salmonella typhimurium. Am. Jour. Vet. Res. 18.432.

Harry, E. G.: 1954. Studies on disinfection of eggs and incubators. IV. The use of ammonia in formaldehyde fumigation practice. Brit. Vet. Jour. 110.380.

——— and Binstead, J. A.: 1961. Studies on disinfection of eggs and incubators. V. The toxicity of formaldehyde to the developing embryo. Brit. Vet. Jour. 117:532.

Harwood, P. D.: 1954. Clinical applications of nitrofurans—Past and present. Proc. 1st Nat. Symposium on Nitrofurans in Agr. P. 12.

Hashimoto, K.: 1961. Studies on the bacterical action of embryonating eggs against Salmonella pullorum and S senftenberg. II. The bactericidal action of various liquid materials of embryonating eggs. Japanese Jour. of Bact. 16(6) 417.

Heemstra, L. C.: 1952. Salmonella infections in chickens and turkeys. Proc. Book Am. Vet. Med. Assn. 89th Ann. Meet. P. 311.

Henderson, W., Ostendorf, J., and Storehouse, G. L.: 1960. The relative pathogenicity of some Salmonella serotypes for chicks. Avian Dis. 4:103.

Hennig, M. W.: 1953. The antigenic structure of salmonellas obtained from domestic animals and birds in South Africa. Onderstepoort Jour. Vet. Sci. and Anim. Ind. 15.79.

Higgins, W. A., Christiansen, J. B., and Schroeder, C. H.: 1944. A Salmonella enteritidis infection associated with leg deformity in chicks. Poultry Sci. 23.510.

Hinshaw, W. R., and McNeil, E.: 1943a. The use of the agglutination test in detecting Salmonella typhimurium carriers in turkey flocks. Proc. 47th Ann. Meet. U.S. Livestock Sanit. Assn. P. 108.

——, and McNeil, E.: 1943b. Salmonella newington infection in turkeys. Poultry Sci. 22:415

——, and McNeil, E.: 1944a. The importance of group agglutinations in pullorum testing programs. Proc. 48th Ann. Meet. U.S. Livestock Sanit. Assn. P. 165.

——, and McNeil, E.: 1944b. Gopher snakes as carriers of salmonellosis and paracolon infections. Cornell Vet. 34:248.

——, and McNeil, E.: 1945. Salmonella types isolated from snakes. Am. Jour. Vet. Res 6:264.

——, and McNeil, E.: 1946a. The occurrence of type 10 paracolon in turkeys. Jour. Bact. 51:281.

——, and McNeil, E.: 1946b. Paracolon type 10 from captive rattlesnakes. Jour. Bact. 51:397

——, and McNeil, E.: 1947. Lizards as carriers of Salmonella and paracolon bacteria. Jour. Bact. 53.715.

——, and McNeil, E.: 1948. Avian salmonellosis; its economic and public health significance Proc. 8th World's Poultry Cong. 1:599.

——, and McNeil, E.: 1951. Salmonella infection as a food industry problem. Advances in Food Res. 3:209.

——, McNeil, E., and Taylor, T. J.: 1944. Avian salmonellosis. Types of Salmonella isolated and their relation to public health. Am. Jour. Hyg. 40-264.

——, Taylor, T. J., and McNeil, E.: 1942. Salmonella bredeney infection in birds. Cornell Vet. 32:337.

Hirsch, W.: 1947. A new bacterial variant: The non-motile H form. Jour. Hyg., Cambridge 45.417.

Hobbs, B. C.: 1961. Public health significance of Salmonella carriers in livestock and birds. Jour. Applied Bact. 24:340.

——: 1963. Techniques for the isolation of Salmonellae from eggs and egg-products. Ann. Inst. Pasteur 104:621.

——, Reeves, J. G.: Carside, J. S., Gordon, R. F., Barnes, E. M., Shrimpton, D. H., and Anderson, E. S.: 1960. Antibiotic treatment of poultry in relation to Salmonella typhi-murium Month. Bul. Minist. Health Lab. Serv. 19:178

Hoffmann, H. A., and Edwards, P. R.: 1937. The spontaneous transmission of IV – Variants of Salmonella oertrycke from pigeons to rabbits. Am. Jour. Hyg. 26-135.

——, Jones, E. E., and Stover, D. E.: 1943. Paratyphoid and paracolon infections in chickens and turkeys. State of Calif. Dept. of Agr. Bul., Vol. 32 66.

Hofmann, P., Horchner, P., and Wolle-Jollen, R.: 1960. Einschleppung Seltener Salmonellen durch importierte Geflugelfedern. Zentralbl. f Bakt. 1 orig. 178:484.

Hohn, J., and Hermann, W.: 1955. Die Typen der Gartnerbakterien und die Quelle ihrer Infektion in der Tierwelt. Zentralbl. f. Bakt. 1 orig. 133:183.

Hole, N.: 1952 Salmonella infections in ducklings. Jour. Comp. Path. and Therap. 45:161

Hudson, C. B.: 1942. An outbreak of paratyphoid in guineas. Jour. Am. Vet. Med. Assn. 100.438

——, and Tudor, D. C.: 1957. Salmonella typhimurium infection in feral birds. Cornell Vet. 47:394,

Huey, C. R., and Edwards, P. R.: 1958. Resistance of Salmonella typhimurium to tetracyclines Proc. Soc. for Exp. Biol. and Med. 97:550.

Huyghelen, C., Mortelmans, J., and Vercruysse, J.: 1958. Infekties door Salmonella saint paul, Salmonella senegal, Salmonella veje, Salmonella infantis, Salmonella braenderup en Salmonella thompson, als oorzaak van kuikensterfte. Vlaams Diergeneesk. Tijdschr. 27:201.

Jamison, S. L.: 1956. Paracolon infection. Pacific Poultryman, March, 1956, p. 40.

Jansen, J.: 1936. Eendenkukensterfte door S. typhi-murium en S. enteritidis var. Essen. Tijdschr. Diergeneesk, 63.140.

Jeffries, L.: 1959. Novo-bioom-tetrathionate broth A medium of improved selectivity for the isolation of Salmonellae from faeces. Jour. Clin. Path. 12.568.

Johnson, S. D., Druner, D. W., and Murphy, J. M.: 1951. Infection of the bovine udder with paracolon bacteria. Cornell Vet. 41:283

Jones, F. S., Orcutt, M., and Little, R. B.: 1932. Atypical (slow) lactose fermenting B. coli Jour. Bact. 23:267.

Jones, T. J.: 1959. The control of game bird diseases Mod. Game Breeding and Hunting Club News 29.12.

Jungeman, P. F., and Grombles, L. C.: 1960. Salmonella organisms in mature, healthy dogs. Southwestern Vet. 13:208.

Jungherr, E., and Clancy, C. F.: 1939. Serological types of Salmonella isolated from paratyphoid in chicks. Jour. Infect. Dis. 64:1.

——, Hall, W. J., and Pomeroy, B. S.: 1940. Techniques for the bacteriologic examination of reactors to pullorum disease antigen. Proc. Book Am. Vet. Med. Assn. 87th Ann. Meet. P. 260.

——, and Wilcox, K. S.: 1934. Salmonella aertrycke as an etiologic agent of paratyphoid in pigeons. Jour. Infect. Dis. 55:590.

Kampelmacher, E. H.: 1963. Salmonellosis in the Netherlands. Ann. Inst. Pasteur 104.617.

Karlhoj, K., and Stabo, L.: 1949. Salmonella infection in eggs of ducks. Am. Jour. Vet. Res 10:588.

Kauffmann, F.: 1941. Über mehrere neue Salmonella-Typen. Acta Path. et Microbiol. Scand. 18:351.

———.: 1945. Personal communication cited by Edwards, West, and Bruner (1947).

———.: 1950. The Diagnosis of Salmonella Types. Charles C Thomas, Springfield, Ill.

———.: 1954. Enterobacteriaceae, 2nd ed. E. Munksgaard, Copenhagen, Denmark.

———.: 1956. Group and type classification of Enterobacteriaceae by biochemical and serological criteria. Zentralbl. f. Bakt. I orig. 165:344.

———, and Edwards, P. R.: 1947. A simplification of the serologic diagnosis of Salmonella cultures. Jour. Lab. and Clin. Med. 32:548.

———, and Edwards, P. R.: 1957. A revised, simplified Kauffmann-White schema. Acta Path. et Microbiol. Scand. 41:2452.

———, and Moeller, E.: 1940. A new type of Salmonella (S. ballerup) with Vi-antigen. Jour. Hyg., Cambridge 40.246.

Kaye, D., Shinefield, H. R., and Hook, E. W.: 1961. The parakeet as a source of salmonellosis in man Report of a case. New Eng. Jour. Med. 264.868.

Keymer, I. F.: 1959. Specific diseases of the canary and other passerine birds. Modern Vet. Pract. 40(17):32.

Khalifa, I. A. B.: 1935 Serological study of pigeon paratyphoid in Egypt. Jour. Am. Vet. Med. Assn. 86.24.

Klatt, C. H.: 1961 Om Salmonellaundersokningar hos fjaderfä f helsingfors. Finsk. Vet. Tidskr. 67:283

Kraft, J. R.: 1951 Paracolon pneumonia. Am. Jour. Clin. Path. 21:666.

Kraus, P., and Weker, G.: 1958. Untersuchungen über die Haltbarkeit von Krankheitserregern in Trink- und Oberflächenwasser. Zentralbl f. Bakt. I orig. 171:509.

Ladehoff, G.: 1955. Beitrag zur Epidemiologie von Salmonella typhi murium in Schleswig-Holstein. Inaug Diss. Hannover

Lahaye, J., and Willems, R.: 1927 Une maladie des pigeons due à un germe du groupe des Salmonella. Ann Méd. Vét 72:211.

Lancaster, J. E.: 1952 A note on the toxicity of formaldehyde to the developing chicken embryo. Canad. Jour. Comp. Med and Vet. Sci. 26 159

———, and Crabb, W. E.: 1953a Studies on disinfection of eggs and incubators. I. The survival of Salmonella pullorum, thompson, and typhi-murium on the surface of hen's egg and on incubator debris. Brit. Vet. Jour. 109.139.

———, and Crabb, W. E.: 1953b. Studies on disinfection of eggs and incubators. II. The value of formaldehyde gas with particular reference to the concentration resulting from the addition of formalin to potassium permanganate. Brit. Vet. Jour. 109.390.

———, Gordon, R. F., and Harry, E. G.: 1954. Studies on disinfection of eggs and incubators. III. The use of formaldehyde at room temperature for the fumigation of eggs prior to incubation. Brit. Vet. Jour 110 238

———, Gordon, R. F., and Tucker, J.: 1952. The disinfection, prior to incubation of hen eggs contaminated with Salmonella pullorum. Brit. Vet. Jour 108·418.

Lannek, N., Lindgren, N. O., and Nilsson, T.: 1962 Therapeutical experiments with a new nitrofuran compound (Tsufur) in salmonellosis of chicks Avian Dis. 6.228.

Lee, C. D.: 1957. Evaluation of the paratyphoid (Salmonella typhimurium) program in Iowa 1956-57. Proc. 6th Ann. No. Central Reg. Poultry Dis. Conf.

———, Holm, G., and Murray, C 1956. Paratyphoid infection in turkeys. Jour. Am. Vet. Med. Assn. 89.65.

Leiche, M.: 1936. Zur Entstehung Bakterieller Lebensmittelschädinigungen durch Geflügel und Geflügelprodukte. Proc. 6th World's Poultry Cong. 1.398.

———: 1953. Salmonellainfektionen beim Geflügel und ihre Bedeutung für die Epidemiologie der Salmonella-Bakterien. Proc. 7th World's Poultry Cong. p. 274.

———: 1957 Über die Abtötung von Salmonella Bakterien im Weissel. Berl. und Münchener tierarztl Wochenschr. 70 436

Levine, N. D., and Graham, R.: 1942. Paratyphoid in baby wood ducks. Jour. Am. Vet. Med. Assn. 100 240.

Lewis, K. H., and Büchner, E. R.: 1953. Slow lactose-fermenting bacteria pathogenic for young chicks. Jour. Infect. Dis 59.225

Lisky, W., Fagerson, I S, and Fellers, C. R.: 1957. A bacteriological survey of commercially frozen beef, poultry and tuna pies. Jour Milk and Food Technol. 20 216.

Lucas, F. R.: 1956 Use of furazolidone in a field outbreak of salmonellosis in mallard ducks Jour. Am. Vet. Med. Assn. 129 529

Luippold, G. F.: 1947. A paracolon organism antigenically related to the Sachs Q-1030 bacillus and associated with chronic enterocolitis. Gastroentero. 8:358.

Lukas, G. N., and Bradford, D. F.: 1954. Salmonellosis in turkey poults as observed in routine necropsy of 1,148 cases. Jour. Am. Vet. Med. Assn. 125.215.

Lutje, F.: 1921. Abort und Infektion der Stuten. Deutsch. tierarztl. Wochenschr. 29:453.

Lystad, A.: 1962. An unusual Paracolobactrum aerogenoides as the cause of nosocomial urinary tract infection Acta Path Microbiol Scand. 54.400

MacDonald, A. D.: 1947. K antigen for the detection of pullorum disease in poultry. Proc. 10th Ann. Conf. Lab. Workers in Pullorum Dis Control.

Mackel, D. C., Galton, M. M., Gray, H., and Hardy, A. V.: 1952. Salmonellosis in dogs. IV. Prevalence in normal dogs and their contacts. Jour. Infect. Dis. 91:15.

——, Payne, F. J., and Pirkle, C. I.: 1959. Outbreak of gastroenteritis caused by S. typhi-murium acquired from turkeys. Pub. Health Rep. 74.746.

Maclaury, D. W., and Moran, A. B.: 1959. Bacterial contamination of hatching eggs. Ky. Agr. Exper. Sta. Bul. 665.

Magwood, S. E., and Annau, E.: 1961. The adsorption of somatic antigens of Salmonella by polystyrene latex particles. Canad. Jour. Comp. Med. and Vet. Sc. 25 69.

Mair, N. S., and Ross, A. I.: 1960. Survival of Salmonella typhimurium in the soil Month. Bul Minist. Health Lab. Serv. 19:39.

Mallmann, W. L., Ryff, J. F., and Matthews, E.: 1942. Studies on the Salmonella group— Methods of isolation and pathogenicity of strains occurring in the intestines of chickens Jour. Infect. Dis. 70.253.

Manninger, R.: 1913. Über eine durch den Bazillus paratyphi B verursachte Infektions-Krank-heit der Finken. Zentralbl. f. Bakt. I orig. 70:12.

——: 1918. Über Paratyphus beim Wassergeflugel. Allatorvosi Lapok, Budapest, p. 165. (Abst. in Jahresbr. Vet. Med. 38:160.)

Marcellus, F. N., Gwatkin, R., and Glover, J. S: 1930. Incubator disinfection in the control of Salmonella pullorum. Proc. 4th World's Poultry Cong., Sect. C, p 401

Maritheedal, H. E.: 1962. Occurrence of Salmonella typhi-murium infections in poultry in Den-mark 1954-1960. Epizootological studies. Proc. 12th World's Poultry Cong. P. 278.

Mazza, G: 1899. Bakteriologische Untersuchungen über einer neuerdings aufgetretene Huhner-epizooue. Zentralbl. f. Bakt. 26:181.

McCarty, R. T.: 1953. Neomycin sulfate treatment of Salmonella derby infection in geese Jour. Am. Vet. Med. Assn. 122:386

McClure, H. E., Eveland, W. C., and Kase, A.: 1957. The occurrence of certain Enterobacteria ceae in birds. Amer. Jour. Vet. Res 18.207.

McCullough, N. B.: 1958. Food in the epidemiology of salmonellosis. Jour. Amer. Dietetic Assn. 34:254.

——, and Eisele, C. W.: 1951a. Experimental human salmonellosis. I. Pathogenicity of strains of Salmonella meleagridis and Salmonella anatum obtained from spray-dried whole egg. Jour. Infect. Dis. 88:278.

——, and Eisele, C. W.: 1951b. Experimental human salmonellosis. II. Immunity studies following experimental illness with Salmonella meleagridis and Salmonella anatum. Jour. Immunology 66:595.

——, and Eisele, C. W.: 1951c. Experimental human salmonellosis. III. Pathogenicity of strains of Salmonella newport, Salmonella derby, and Salmonella bareilly obtained from spray-dried whole egg. Jour. Infect. Dis. 89.209.

——, and Eisele, C. W.: 1951d. Experimental human salmonellosis. IV. Pathogenicity of strains of Salmonella pullorum obtained from spray-dried whole egg. Jour. Infect. Dis 89:259.

McElrath, H. B., Galton, M. M., and Hardy, A. V.: 1952. Salmonellosis in dogs. III. Prevalence in dogs in veterinary hospitals, pounds, and boarding kennels. Jour. Infect. Dis. 91:12

McGaughey, C. A.: 1932. Bacteria of the enteric group among poultry. Brit. Vet. Jour. 88:16

McNeil, E., and Hinshaw, W. R.: 1944. Snakes, cats, and flies as carriers of Salmonella typhi-murium. Poultry Sci. 23.456.

—— and Hinshaw, W. R.: 1946. Salmonella from Galapagos turtles, a Gila monster and an Iguana. Am. Jour. Vet. Sci. 7:62.

—— and Hinshaw, W. R.: 1951. Procedures for conducting the agglutination test for de-tection of Salmonella carriers in turkey flocks. Vet. Med. 46:360.

Meyer, K. F.: 1942. Ecology of psittacosis and ornithosis. Medicine 21:175.

——, and Eddie, B: 1934. Latent psittacosis and Salmonella psittacosis infection in South American parrotlets and conures. Science 79.546.

——, and Eddie, B: 1939. Psittacosis in importations of psittacine birds from South American and Australian commerce. Jour. Infect. Dis. 65:234.

Milner, K. C., and Shaffer, M. F.: 1952 Bacteriologic studies of experimental Salmonella in-fections in chicks. Jour. Infect. Dis. 90.81.

Mitrovic, M.: 1956. First report of paratyphoid infection in turkey poults due to Salmonella reading. Poultry Sci. 35:171.

——, Mailsheck, P. H., and Lynch, L. C.: 1961. The chemotherapeutic activity of 3, 5-dinitrobenzamide. 1. Against paratyphoid in turkeys. Avian Dis. 5.5

Mohler, J. R.: 1904. Infectious enteritis of pigeons. Ann. Rep. of Bur. Anim. Ind., U.S.D.A. p 26.

Moore, V. A.: 1895. On a pathogenic bacillus of the hog-cholera group associated with a fatal disease in pigeons Bur. Anim. Ind., U.S.D.A., Bul. 8.71.

Moran, A. B: 1959a. Salmonella in animals A report for 1957. Avian Dis 3:85

Moran, A. B.: 1959b. Serotypes of Salmonella and Arizona organisms in animals: 1958. Avian Dis. 3:440.

———: 1960. Salmonella and Arizona cultures of animal origin: 1958. Avian Dis. 4:73.

———: 1961a. Salmonella and Arizona cultures from agricultural sources: 1959. Avian Dis. 5:147.

———: 1961b. Occurrence and distribution of Salmonella in animals in the United States. Proc. 65th Ann. Meet. U.S. Livestock Sanit. Assn. P. 441.

——— and Bruner, D. W.: 1949. Further studies on the Bethesda group of paracolon bacteria. Jour. Bact. 58:695.

Morcos, Z.: 1935. Pigeon paratyphoid. Brit. Vet. Jour. 91:11.

Morehouse, L. G., and Wedman, E. E.: 1961. Salmonella and other disease producing organisms in animal by-products—A survey. Jour. Am. Vet. Med. Assn. 139.989.

Morris, T. G., and Ayres, J. C.: 1960. Incidence of Salmonellae on commercially processed poultry. Poultry Sci. 39:1131.

Mortelmans, J., Huygelen, C., and Vercruysse, J.: 1958. Le transport de poussins par avion, moyen de dispersion des Salmonellae. Bul. Soc. Path. Exot. 51:294.

Muller, J.: 1957a. Om salmonellainfektioner hos svinemeglet. Medlemsbl. danske Dyrlaege-foren 40 631.

———: 1957b. Le problème des Salmonelloses au Danemark. Bul. mensuel. Off. Internat. Epizoot., Paris 48:523.

———: 1961. Om forekomsten af Salmonellose hos fjerkrae. Nordisk Veterinaermed. 13:617.

Mundt, J. O., and Tugwell, R. L.: 1958. The relationship of the chicken egg to selected paratyphoids. Poultry Sci. 37:415

Mushin, R.: 1949. Studies on paracolon bacilli. Australian Jour. Exp. Biol. and Med. 27.543.

Nakamura, N., Nose, Y., and Negishi, B.: 1939. An outbreak of Salmonella enteritidis infection in baby turkey poults. Proc 7th World's Poultry Cong. P. 240.

Newell, K. W., Hobbs, B. C., and Wallace, E. J. G.: 1955. Paratyphoid fever associated with Chinese frozen whole egg. Brit Med Jour. 2 Pt. 2:1296.

———, McClarin, R., Murdock, C. R., Macdonald, W N., and Hutchinson, H. L.: 1959. Salmonellosis in Northern Ireland, with special reference to pigs and Salmonella contaminated pig meal. Jour. Hyg., Cambridge 57:92.

Niemeyer, W. E.: 1939. Paratyphoid and trichomonas infection in pigeons. Jour. Am. Vet. Med Assn. 94:434.

Niven, C. F.: 1901 Industry's role in reducing the incidence of Salmonella in animal feeds. Proc 65th Ann. Meet, U.S Livestock Sanit. Assn. P. 453.

Olson, B. H., and Jennings, J. C.: 1954. Effect of synemamin B treatment of Salmonella infections in mice and chicks. Antibiotics and Chemotherapy 4.11.

Ono, T., Kato, E., Lee, S. T., Hamada, S., Hirato, K., Fukumi, H., and Sakaguchi, G.: 1953. Studies on chick salmonellosis. 1 Bacteriological observation on dead embryos and brooding chicks. Vet. Res (Japan) 1:61.

Osborne, W. W., and Stokes, J. L.: 1955 A modified selenite brilliant-green medium for the isolation of Salmonella from egg products Applied Microbiology 3:295.

Ostrolenk, M., and Welch, H.: 1942. The house fly as a vector of food poisoning organisms in food producing establishments. Am. Jour. Pub. Health 32.487.

Pampana, E. J.: 1933 Microbic dissociation Detection of the "R" variant by means of a specific drop agglutination Jour. Hyg. Cambridge 33:402.

Papadakis, J A.: 1900. Dulcitol-sucrose-salicin iron-urea agar (DSSIU)—A new medium for differential diagnosis of Salmonellae. Jour Hyg., Cambridge 58 331.

Paul, H. E.: 1956. Research background on the nitrofurans. Proc. 1st Nat. Symposium on Nitrofurans in Agr P 6

Peluffo, C. A., Edwards, P. R., and Bruner, D. W.: 1942. A group of coliform bacilli serologically related to the genus Salmonella. Jour. Infect. Dis. 70:185.

Perek, M.: 1957. Isolation of a Paracolobactrum organism pathogenic to chicks. Jour. Infect Dis. 101:8.

——— and Rabinowitz, S: 1957. The value of faeces examinations in detection of carriers of salmonellosis in geese. British Vet. Jour. 113:511.

Perelli-Minetti, J E, Rucker, J. C, and Ross, F. K.: 1948. Paratyphoid. Calif. Dept. Agr. Bul 37(No. 3):151.

Peterson, E. H.: 1947. Field studies of sulfamerazine in the control of pullorum and some other diseases of domestic birds. No. Am. Vet. 28:293.

Petzelt, K., and Stelinger, R.: 1954. Die Vogel der Klaranlage von Hannover und die von ihnen Ausgeschiedenen Salmonellen. Arch. Hyg. und Bakt. 145.605.

Pfaff, Fr.: 1921. Eine Truthhuhnerseuche mit Paratyphus-Befund. Zeitschr. f. Infekt -Krankh. d. Haustiere 22.285.

Pfeiler, W.: 1920. Beitrag zur Kasustik des Huhnertyphus. Zeitschr. f. Fleisch-und Milch-Hyg 30.267.

———, and Rehse, A.: 1913 Ueber das Vorkommen von Bakterien aus der Gruppe der Fleischvergifter bei Vogeln. Paratyphus B-Infektion beim Huhn. Zentralbl. f. Bakt. 1 orig 68:174.

Philbrook, F. R., MacCready, R. A., Van Roekel, H., Anderson, E. S, Snyser, C. F., Sanen, F. J., and Groton, W, M.: 1960. Salmonellosis spread by a dietary supplement of avian source. New Eng. Jour. Med. 263:713.

Pomeroy, B. S.: 1944. Salmonellosis of turkeys. Doctoral thesis Univ. Minn.
——: 1938. The control of paratyphoid infections and typhumurium testing programs. Rep. of Nat. Plans Conf., U.S.D.A., ARS, AHR Division, Beltsville, Md., p. 15.
——, Belding, R. C., Williams, J. E., Erwin, L. E., Vickers, G. S., and Moran, A.: 1957b. Report of the committee on Salmonella (1957) for N.C. regional poultry disease conference. Proc. 8th Ann. No. Central Reg. Poultry Dis. Conf.
——, and Fenstermacher, R.: 1939. Paratyphoid infection of turkeys. Jour. Am. Vet. Med. Assn. 94:90.
——, and Fenstermacher, R.: 1941. Paratyphoid infections of turkeys. Am Jour. Vet. Res 2:285.
——, and Fenstermacher, R.: 1943 Salmonella infections of breeding turkeys. Am. Jour Vet. Res. 4:199.
——, and Fenstermacher, R.: 1944. Salmonella infections in turkeys. Am. Jour. Vet. Res 5.282.
——, Fenstermacher, R., and Roepke, M. H.: 1948. Sulfonamides in the control of salmonellosis of chicks and poults. Jour. Am. Vet. Med. Assn. 112 206
——, Fenstermacher, R., Jones, F., and Jenkins, L. E.: 1957a. The control of paratyphoid and related enteric infections of turkeys. Proc. 8th Ann. No. Central Reg. Poultry Dis Conf.
——, and Grady, M. K.: 1961. Salmonella organisms isolated from feed ingredients. Proc 65th Ann. Meet. U.S. Livestock Sanit. Assn. P. 449.
——, Juhl, J. R., and Tumlin, J. T.: 1958. Arizona-type paracolon infection of turkeys. Proc. 2nd Nat. Symposium on Nitrofurans in Agr., Hess and Clark, Inc., Ashland, Ohio.
Poedl, J. J.: 1942. Intestinal cultures for detecting salmonellosis in young turkeys. Am. Jour Vet. Res. 3:257.
Price, J. I., Dougherty, E., and Bruner, D. W.: 1962. Salmonella infections in White Pekin duck. A short summary of the years 1950-60. Avian Dis. 6.145
Pulst, H.: 1960. Ein Beitrag zur Salmonellose beim Wassergeflugel. Mh. Vet. Med. 15.226.
Quesada, A., Izzi, R., and Maggio, V.: 1960. Sulla presenza di germi del genere Salmonella nelle farine di pesce impiegate per la confezione dei mangimi. Soc. Italian delle Sci. Vet. Atti. 14:757.
Quist, K. D.: 1962. Salmonella in poultry as related to human health. Rep. of Nat. Plans Conf. U.S.D.A., ARS, AHR Division, Beltsville, Md., p. 24.
Ramsey, C. H., and Edwards, P. R.: 1961. Resistance of Salmonellae isolated in 1959 and 1960 to tetracylines and chloramphenicol. Applied Microbiology 9.389.
Rao, S. B. V.: 1956. Isolation of Salmonella hichfield from an outbreak in chicks in the Indian Union. Indian Jour. Vet. Sci. 26:131.
——, and Gupta, B. R.: 1961. The isolation of Salmonella weltevreden and Salmonella dublin in an outbreak of salmonellosis in imported chicks. Indian Jour. Med. Res. 49 6
Rasmussen, P. G.: 1962. Salmonella typhimurium-ledbetaendelse hos slagteaender. Ledbetaendelsernes aetiologi og fjerkraekontroimaessige bedrmmelse. Nordisk Veterinaermed. 14:99.
Ravaioli, L., and Oriel, Z.: 1952. La cloromicetina e la teramicina nella terapia della Salmonellusi aviare. Soc. Italian delle Sci. Vet. Atti. 6-936.
Reuger, L. F., Plastridge, W. N., and Cameron, R.: 1933. Endemic paratyphoid infection in turkeys. Jour. Infect. Dis. 53-272.
——, and Scoville, M.: 1920. Bacterium anatum n. s. the etiologic factor in a widespread disease of young ducklings known in some places as "keel." Jour. Infect. Dis. 26.217.
Rice, C. E., Magwood, S. E., and Annau, E.: 1960. A modified direct complement-fixation test for the detection of antibodies for Salmonella antigens in turkey sera. Canad. Vet. Jour. 1:132.
Ryff, J. F., and Browne, J.: 1952. Paracolon abortion in ewes. Jour. Am. Vet. Med. Assn 121:266.
Sadler, W. W., Yamamoto, R., Adler, H. E. and Stewart, G. F.: 1961. Survey of market poultry for Salmonella infection. Applied Microbiology 9.72.
Sakazaki, R.: 1951. A Salmonella type "S. essen" bacillus isolated from a fatal case of fowl paratyphoid with special reference to its serological variation. Japanese Jour. of Vet. Sci 13 (No. 3):121.
——, Shigeo, N., and Watanabe, S.: 1959. The occurrence and distribution of Salmonella and Arizona in Japan. Japanese Jour. Exper. Med. 29:15.
Salisbury, R. M.: 1958. Salmonella infections in animals and birds in New Zealand. New Zeal. Vet. Jour. 6:76.
Sanders, R. G., Pomeroy, B. S., and Fenstermacher, R.: 1943. Cross-agglutination studies between Salmonella pullorum and other microorganisms isolated from turkeys positive to the pullorum test. Am. Jour. Vet. Res. 4:194.
Savage, W.: 1956. Problems of Salmonella food poisoning. Brit. Med. Jour, Aug. 11, 1956, p. 317.

Schaaf, J.: 1936. Remarks made during discussion of paratyphoid infections of poultry. Proc 6th World's Poultry Cong. 3 409.

Schalm, O. W.: 1937. Study of a paratyphoid infection in chicks. Jour. Infect. Dis. 28:43.

Schiff, F., Bornstein, S., and Saphra, I.: 1941. The occurrence of Salmonella O-antigens in coliform organisms. Immunology 40.365.

Schneider, M. D.: 1946. Investigation of Salmonella content of powdered whole egg with not more than 25% moisture content. II. General survey on the occurrence of species of Salmonella in high quality egg powder. Food Res 11.515.

——, and Gunderson, M. F.: 1949 Investigators shed more light on Salmonella problem. U.S. Egg and Poultry Magazine 55:10.

Schoop, G., and Mosez, K.: 1954. Zur Therapie der Salmonellen-Infektion (Flugellähme) der Tauben. Berl. und Munchener tierarztl Wochenschr. 67:53.

Schwerin, K O.: 1960. Möwan als Salmonellenausscheider und ihre Bedeutung fur die Verseuchung von Inlandischen Freilandbrut Mh. Vet. Med. 15:377.

Sedlmeier, H., Kotter, L., and Terplan, G.: 1957. Zum Vorkommen von nicht Huhnerspezifischen Salmonellen bei Huhnern. Berl. und Munchener tierarztl. Wochenschr. 70:433.

Shaffer, M. F., Milner, K. C., Clemmer, D I., and Bridges, J. F.: 1957. Bacteriologic studies of experimental Salmonella infections in chicks. II. Jour. Infect. Dis. 100.17.

——, Milner, K. C., Clemmer, D I., Potash, L., and Shaffer, L. S.: 1954. WN 5063–2 (Thiocymetin). A bacteriologic evaluation of its therapeutic effectiveness against experimental Salmonella infections Antibiotics and Chemotherapy 4:992.

Shirlaw, J. F., and Iyer, S G.: 1937. A note on a variety of S. enteritidis isolated from pigeons. Indian Jour, Vet. Sci. and Anim Husb. 7:231.

Sieburth, J. McI.: 1957a. The effect of furazolidone on the cultural and serological response of Salmonella typhi-murium infected chickens. Avian Dis. 1:180.

—— . 1957b A procedure for the differentiation of Salmonella and Arizona groups from other enteric organisms Avian Dis. 1.348.

—— . 1957c. Indirect hemagglutination studies on salmonellosis of chickens. Jour. Immunology 78.380.

—— . 1958a. Respiratory flora and diseases of Antarctic birds Avian Dis, 2:402.

—— . 1958b The indirect hemagglutination test in the avian Salmonella problem. Am. Jour Vet Res 19.729.

—— . 1960. Stable, standardized, sensitized chicken erythrocytes for the polyvalent indirect hemagglutination test Am. Jour. Vet. Res. 21:1084.

——, and Johnson, E. P 1956. Observations on stress factors and serological response in Salmonella typhi-murium infection of chicks. Proc. 28th Ann. Conf. Laboratory Workers in Pullorum Disease Control

Simakaya, A M.: 1955. Gigienicheskaya otsenka teplovoi obrabotki utinykh yaits, Voprosy Pitanna 14 54.

Slavkov, I.: 1961 Resistance des Salmonella dans le sol. Proc. 2nd Symp. Int. Assn. Vet. Food Hyg (Basel) P. 279

Smith, H. W: 1955. The treatment of experimental Salmonella typhimurium infection in turkey poults and chicks Vet. Record 67.749.

—— . 1959. The isolation of Salmonella from the mesenteric lymph nodes and faeces of pigs, cattle, sheep, dogs, and cats and from other organs of poultry. Jour. Hyg., Cambridge 57:266

Smyser, C F , and Van Roekel, H 1959. Detection of Salmonella typhi-murium infection in an egg producing chicken flock. Avian Dis 3:485.

Solowey, M., Sutton, R R., and Calesnick, E. J.: 1946. Heat resistance of Salmonella organisms isolated from spray-dried whole egg powder. Food Res. 11:580.

——, McFarland, W H, Spaulding, E H., and Chemerda, G.: 1947. Microbiology of spray-dried whole egg. II. Incidence and types of Salmonella. Am Jour. Pub. Health 37.971.

Spink, M S.: 1960 Brucliers. Lancet, September 24, p. 707.

Spray, R. S., and Doyle, L. P.: 1921. Paratyphoid bacilli from chicks. Jour. Infect. Dis. 28:43.

Steiniger, F.: 1961 Wie Lange Halten sich Salmonellen aus Verregneten Abwasser auf Pflanzen? Berl und Munchener tierarztl. Wochenschr. 74:589.

Stokes, J. L., and Osborne, W. W.: 1955. A selenite brilliant green medium for the isolation of Salmonella. Applied Microbiology 3:277.

——, Osborne, W. W., and Bayne, H. G.: 1956. Penetration and growth of Salmonella in shell eggs. Food Res. 21:510.

Stone, R. M.: 1960. Pet bird practice. Jour. Am. Vet Med. Assn. 157:364.

Stover, D, F.: 1960. Fumigation of hatching eggs State of Calif. Dept. of Agr. Bul., Vol. 49 30

—— . 1961. The Salmonella Infections (pullorum, typhoid, and paratyphoid). U.S D.A . ARS 45-2, Disease, Environmental, and Management Factors Related to Poultry Health, p. 45

Strauss, J, Bedrir, B., and Sery, V: 1957 The incidence of ornithosis and salmonellosis in the black-headed gull (Larus ridibundus L.). II. Isolation and identification of the virus of ornithosis from the black-headed gull with simultaneous isolation of S. (Salmonella) typhi-murium. Jour. Hyg. Epidemiol., Microbiol., and Immunol. (Prague) 1.250.

Stuart, C. A., Van Stratum, E., and Rustigian, R.: 1945. Further studies on urease production by Proteus and related organisms. Jour. Bact. 49 437

———, Wheeler, K. M., Rustigian, R., and Zimmerman, A.: 1943. Biochemical and antigenic relationships of the paracolon bacteria. Jour. Bact. 45 101.

Stucker, C. L., Galton, M. M., Cowdery, J., and Hardy, A. V.: 1952. Salmonellosis in dogs. II. Prevalence and distribution in greyhounds in Florida. Jour. Infect. Dis. 91·6.

Sylvester, K.: 1961. Możliwość zakażania środowiska pat eczkami z grupy Salmonella przez formy rozwojowe *Calliphora erythrocephala* meig. Weterynaria Wrocław 9.9.

Tailpour, J. M., and Avery, R. J.: 1960. A survey of turkey viscera for Salmonellae. British Columbia. Canad. Jour. Pub. Health 51:75.

Taylor, J.: 1960. Food poisoning. (b) Salmonella and salmonellosis. Roy. Soc. Health Jour. 80:255.

———: 1963. The serotypes of Salmonella isolated from foods. Ann. Inst. Pasteur 104:660.

Thatcher, F. S., and Montford, J.: 1962. Egg products as a source of Salmonellae in processed foods. Canad. Jour. Pub. Health 53:61.

Thomas, J.: 1961. Salmonelles isolées en Belgique chez les animaux et dans les denrées alimentaires d'origine animale. Ann. Méd. Vét. 105.206.

Trawiński, A., and Trawińska, J.: 1960. Badania nad przenoszeniem Salmonel przez muchy w stadiach rozwojowych. Ann. Univ. M. Curie-Skłodowska, Sect. DD 15·31.

Truscott, R. B.: 1956. *Salmonella moscow* isolated from ducks in Ontario. Canad. Jour. Comp. Med. and Vet. Sci. 20:515.

Vallée, A., Le Minor, L., Collin, P., and Girardin, A.: 1956. Enzootie de paratyphose à *Salmonella johannesburg* chez des bengalis. Rec. Méd. Vét. Ecole d'Alfort 135:585.

Van Dorssen, C. A.: 1955. *Salmonella typhi-murium* Typen uit Duiven. Tijdschr. Diergeneesk 80:1188.

Van Keulen, A.: 1959. Salmonellosis als Zoönose. Tijdschr. Diergeneesk. 84:1102.

Van Roekel, H., and Bullis, K.: 1937. Salmonella infections in chickens. Jour. Am. Vet Med. Assn. 91:48.

Walker, J. H. C.: 1960. The broiler industry–Transmission of Salmonella infection. Roy. Soc Health Jour. 80.142.

Warrack, G. H., and Dalling, T.: 1933. Salmonella infections in young ducklings and duck eggs. Brit. Vet. Jour. 89.483.

Watanabe, S., Hashimoto, K., and Kume, T.: 1958. Studies on Salmonella infection in hen's eggs during incubation with special reference to the mode of infection with *S pullorum* and *S. senftenberg*. II. Transmission of Salmonella from infected hens to laid eggs Bul. Nat. Inst. Anim. Health (Tokyo) 36:1.

———, Hashimoto, K., Kume, T., Murata, M., and Sakazaki, R.: 1959a. Studies on Salmonella infection in hen's eggs during incubation with special reference to the mode of infection with *S. pullorum* and *S. senftenberg*. III. Salmonella infection of embryonated eggs through the egg shell. Bul. Nat. Inst. Anim. Health (Tokyo) 37:47.

———, Kume, T., Hashimoto, K., and Sakazaki, R.: 1959b. Studies on Salmonella infection in hen's eggs during incubation with special reference to the mode of infection with *S. pullorum* and *S. senftenberg*. IV. Natural resistance of chick embryos against *S. pullorum* and *S. senftenberg*. Bul. Nat. Inst. Anim. Health (Tokyo) 37:61.

Watkins, J. R., Flowers, A. I., and Grumbles, L. C.: 1959. Salmonella organisms in animal products used in poultry feeds. Avian Dis. 3:290.

Watt, J.: 1945. An outbreak of Salmonella infection in man from infected chicken eggs. Pub. Health Rep. 60:835.

Watts, P. S., and Wall, M.: 1952. The 1951 *Salmonella typhi-murium* epidemic in sheep in South Australia. Australian Vet. Jour. 28:165.

Wedman, E. E.: 1961. Findings and recommendations of the United States Department of Agriculture task force on Salmonella in animal by-products and feeds. Proc. 65th Ann. Meet. U.S. Livestock Sanit. Assn. P. 458.

Weisgerber, and Muller, C.: 1922. Untersuchungen über eine seuchenhafte Erkrankung der jungen Ganse in der Provinz Ostpreussen mit Paratyphus Befund. Deutsch. tierarztl Wochenschr. 30.663.

West, M. G., and Edwards, P. R.: 1954. The Bethesda-Ballerup group of paracolon bacteria Pub. Health Monograph No. 22. U.S. Pub. Health Serv. Publ. No. 362.

White, P. B.: 1929. Notes on intestinal bacilli with special reference to smooth and rough races. Jour. Path. and Bact. (Scotland) 32:85.

Williams, J. E.: 1965. Unpublished data.

———, and MacDonald, A. D.: 1955. The past, present, and future of Salmonella antigens for poultry. Proc. South. Pac. Vet. Med. Assn. 92nd Ann. Meet. P. 333.

Williams, R. B., and Dodson, M. W.: 1960. Salmonellosis in Alaska. Pub Health Rep 75 913.

Williams, W. L., and Lewis, R. H.: 1961. Prevalence of Salmonellae in meat and poultry products. Jour. Infect. Dis. 109:166.

Wilson, E., Paffenbarger, R. S., Foter, M. J., and Lewis, J. E.: 1944. Observations on fowl paralysis and some current conditions in poultry problems. Vet. Record 56.521

Wilson, J. E.: 1945. Infected egg shells as a means of spread of salmonellosis in chicks and ducklings. Vet. Record 57:441.

———: 1947. The isolation of S. typhi-murium from fowls which gave a positive agglutination test with S. pullorum antigen. Brit. Vet. Jour. 103:101.

———: 1948. Avian salmonellosis. Vet. Record 60:615.

———: 1949. The control and prevention of infectious disease in the hatchery. Brit. Vet. Jour. 105:463.

———: 1950. The occurrence of S. typhi-murium in hen eggs and its implications. Vet. Record 62:449.

———: 1951. The control of salmonellosis in poultry with special reference to fumigation of incubators. Vet. Record 63:501.

———: 1955. The use of furazolidone in the treatment of infections of day-old chicks with S. pullorum, S. gallinarum, S. typhi-murium, and S. thompson. Vet. Record 67:849.

Wolff, A. H., Henderson, N. D., and McCallum, G. L.: 1948. Salmonella from dogs and the possible relationship to salmonellosis in man. Am. Jour. Pub. Health 38:403.

Wolfgang, R. W.: 1958. Recent progress in nitrofuran research. Proc. 2nd. Nat. Symposium on Nitrofurans in Agr. P. 14.

Wright, G. W., and Frank, J. F.: 1956. Penetration of eggs by Salmonella typhi-murium. Canad. Jour. Comp Med. and Vet Sci 20:453.

Yamamoto, R., Adler. H. E., Sadler, W. W., and Stewart, C. F.: 1961a. A study of Salmonella typhimurium infection in market-age turkeys. Am. Jour. Vet. Res. 22:382.

———, Kilian, J. G., Babcock, W. E., and Dickinson, E. M.: 1962. Some observations on serological testing for Salmonella typhimurium in breeder turkeys. Avian Dis. 6:444.

———, Sadler, W W., Adler, H. E., and Stewart, C. F.: 1961b. Comparison of media and methods for recovering Salmonella typhimurium from turkeys. Applied Microbiology 9:76.

Yurchenco, J. A., Yurchenco, M. G., and Piepoli, C. R.: 1953. Antimicrobial properties of furoxone (N-5-Nitro-2-furfurylidene-3-amino-2-oxazolidone). Antibiotics and Chemotherapy 3:1035.

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10

Fowl Typhoid

Fowl typhoid is a septicemic disease of domesticated birds. The course may be acute or chronic. The mortality may be moderate or very high, depending largely on the virulence of the inciting organism, *Salmonella gallinarum*. It appears to be primarily a disease of chickens, but in exceptional cases ducks, turkeys, pheasants, peacocks, guineas, and a few other birds are attacked.

History. In 1888 a chicken breeder in England lost 400 chickens as a result of an infectious disease which was at first considered to be fowl cholera. Two hundred of these birds died in the first two months of the outbreak. Specimens were sent to Klein (1889) for necropsy and diagnosis. He reported it chiefly as an infectious enteritis. The intestinal mucosa and serosa were inflamed, and the feces appeared thin and greenish yellow. The spleen was enlarged two to three times; the liver was also somewhat enlarged, soft, flabby, and moist. The cause was an organism which he

named *Bacillus gallinarum*. The same year he reported the disease among grouse, and in 1893 a similar disease among pheasants. The disease was investigated by Smith in Rhode Island in 1894 and more fully by Moore in Virginia and Maryland in 1895. Moore (1895) described the disease as "infectious leukemia" and named the organism *Bacillus sanguinarium*.

Klein observed small numbers of bacilli in the blood. They were nonmotile, Gram-negative, and were easily cultivated. Chickens inoculated subcutaneously became sick in 5 to 6 days and died 2 to 3 days later. A similar disease was described by Lucet (1891) in France. Lignières and Zabala (1905) described a disease which was probably identical with Klein's disease. The catarrhal enteritis and the swollen spleen attracted attention; Gram-negative bacilli were observed in the blood. They were different from those described by Klein in that they first coagulated, and later peptonized, milk with an alkaline reaction.

[329]

Curtice (1902) studied the disease in Rhode Island and named it "fowl typhoid." The disease has been found in Germany, Hungary, Austria, France, Holland, and North and South America, as well as in Algiers. In Germany it was observed by Pfeiler and Rehse (1913) Van Straaten and te Hennepe (1918) in Holland described the disease very fully.

On the basis of the post-mortem observation, Klein believed that it was not cholera, but a special disease. His suspicion was soon confirmed, because he ascertained that the newly discovered organism was different morphologically and biologically from that of fowl cholera.

Transmission. Like most other bacterial diseases, fowl typhoid is spread in several ways. Research on the transmission of fowl typhoid indicates that the infected bird, the reactor and carrier, is by far the most important means of perpetuating and spreading the disease. Such birds may infect not only their own generation but succeeding generations through egg transmission.

Evidence of egg transmission of *S. gallinarum* was reported by Beaudette (1925, 1930), and by Beach and Davis (1927).

Nobrega and Bueno (1942) cultured 1,465 fresh, infertile eggs from 52 hens shown to be chronic carriers of fowl typhoid. These were reactors from three different flocks where severe outbreaks of fowl typhoid among chicks had been experienced. The incidence of *S. gallinarum* in the eggs from the three lots of fowls was 2.8, 0, and 1.73 per cent, respectively.

Moore (1946a) recovered *S. gallinarum* from 8.9 per cent of 395 eggs cultured from a pen of 21 fowl typhoid reactors, some naturally and some artificially infected. He also conducted fowl typhoid transmission experiments with flies, turkey buzzards, rats, and also by mating and by air currents. No evidence was produced to indicate transmission of the disease by flies, by mating, or by air currents. Rats and turkey buzzards were found capable of transmitting fowl typhoid. Boney (1947) isolated *S. gallinarum* from one turkey egg

of 374 cultured from a flock of pullorum reactors.

Gordeuk et al. (1949) found that fowl typhoid is transmitted from artificially infected birds to normal fowls by cohabitation. The mortality among normal birds was 60.9 per cent in one group and 45.8 per cent in another. In other exposure trials by contamination of feed or drinking water with a broth culture of *S. gallinarum* or with feces from fowl typhoid infected hens, mortality among normal pullets varied from 31.8 per cent to 69.6 per cent in four experiments with duplicate groups.

After culturing over 10,000 eggs from two flocks, one naturally infected and one artificially infected, Hall et al. (1949a) found that 50 per cent of typhoid reactors laid infected eggs, and an average of 6 per cent of all unhatched eggs were infected with *S. gallinarum*. That these infected eggs laid by typhoid carriers are highly virulent and may be the means of starting new outbreaks of fowl typhoid in laying flocks is indicated by six feeding trials in which 27 birds ranging in age from eight weeks to one year were fed one or more infected eggs mixed in their mash, with death resulting in all but six in an average period of ten days. These investigators also report that of 906 chicks hatched from these reactors 296, or 32.6 per cent, died of the disease during the first six months with the heaviest loss in the first month.

Rao et al. (1952) recovered *Salmonella gallinarum* from 13 of 36 (36 per cent) eggs from a reactor flock.

Jordan (1956a) recovered, by a single swab, *Salmonella gallinarum* from the fresh feces of 4 of 13 birds acutely ill of fowl typhoid. Single cloacal swabs were positive in 15 of 47 (31.9 per cent) similar birds and were positive in blood cultures in 44 of 47 (93.6 per cent). A total of 377 cloacal swabs were taken at intervals from 36 reactors which had recovered from fowl typhoid 3 to 18 months previously, but only one bird was positive for *S. gallinarum*.

Jordan (1956b) reported isolation of

Salmonella gallinarum from eggs laid by reactors to the rapid blood test:

From 3 of 23 eggs (13 per cent) from 4 recovered birds.

From 13 of 274 eggs (4.75 per cent) from 10 naturally recovered birds.

From none of 217 eggs from 4 birds, treated with Chloromycetin, that had recovered from fowl typhoid.

From 25 of 226 eggs (11 per cent) from 2 recovered birds that received nitrofurazone.

Both *S. pullorum* and *S. gallinarum* were isolated from different eggs from one bird.

Attendants, feed dealers, chicken buyers, and visitors who travel from house to house and from farm to farm may carry the infection unless precautions are taken to disinfect footwear, hands, and clothing. Trucks, crates, and feed sacks are also important. Wild birds, animals, and flies may be important mechanical spreaders, especially if they have been feeding on carcasses of dead birds, or on offal from packing plants or hatcheries.

Distribution. The disease is widespread in the poultry-producing areas of the country, but outbreaks are sporadic, depending on factors which are not completely recognized at present. The distribution changes from year to year and season to season, although the seasonal variation seems to be more marked in the northern than in the southern parts of this country.

In a nationwide survey Moore (1916b) found fowl typhoid to be most prevalent in the eastern states. Nineteen states reported fowl typhoid to be increasing during the preceding five-year period. This was especially noticeable in the Atlantic seaboard states. The survey showed fowls of all ages and breeds to be susceptible, including chickens, turkeys, and guineas. Moore also found fowl typhoid to be most prevalent in Delaware in the summer and fall. Twenty-one states in the survey reported similar observations. Two states reported it to be more common in winter, while eight states had not found the disease to be seasonal. Hall *et al.* (1919a) found that the curve of fowl typhoid infected egg production varied directly with the total egg production and for that reason outbreaks of fowl typhoid tended to be seasonal.

According to reports from Europe, it is now more widespread than before the first World War, although there has been a steady decline in incidence during the past ten years. Truche (1923), in France, and te Hennepe and van Straaten (1921), in Holland, report the disease as most prevalent in the spring and early summer.

According to Hall (1916), fowl typhoid has become much more prevalent in eastern United States during the past few years. It is especially severe in some broiler-raising areas. He reports that birds of all ages, from baby chicks to breeding hens, are affected, and the disease occurs with equal frequency in young and mature stock. Losses range in outbreaks from an occasional bird in old breeding flocks up to 75 per cent or more in younger fowls.

Glover and Henderson (1946) report an outbreak in chickens in Canada and state that it is believed to be the first case reported in that country.

Bigland (1951) reported an increase of fowl typhoid in Alberta from two cases in 1918 to 37 cases in 1952 in the east central part of the province. The spread was attributed largely to poor sanitation in poultry management.

Etiology. The causative agent of fowl typhoid is a relatively short, plump rod about 1.0–2.0μ long and 1.5μ in diameter. It has received the following names: *B. gallinarum, B. sanguinarium, B. typhi gallinarum alcalifaciens, B. paradysenteriae gallinarum, Eberthella sanguinaria, Shigella gallinarum,* and *Salmonella gallinarum.*

The bacilli mostly occur singly but are occasionally united in pairs. They have a tendency to stain a little heavier at the poles than in the center. They are Gramnegative, form no spores and no capsules, grow aerobically, and are nonmotile.

Gelatin colonies: Small, grayish-white, entire.

Gelatin stab: Slight, grayish-white sur-

TABLE 10.2
SUMMARY OF CHARACTERISTICS OF VARIANTS OF *Salmonella pullorum*
AND *Salmonella gallinarum* (HINSHAW, 1941)
Fermentation Reactions

Variants	Maltose	Xylose	Dulcite	Arabinose	Cysteine-Gelatin	Tartrate Agar (J-H)
S. pullorum (Van Roekel) .	AG*	AG	—	AG	—	—
S pullorum (9 Calif. cult.)	AG*	AG	—	‡ or AG	—	—
S. intermedius A-type (4 cult.) . . .	‡ or AG	AG	AG	AG	—	—
S intermedius B-type (2 cult.) . .	A	—	A	A	—	—
S. gallinarum (Kujiungieff)	A	—	—	A	—	A
S. gallinarum (Barboni) . .	—	A	A	A	—	A
S. gallinarum (Van Roekel)	—	A	A	A	T	A
S. gallinarum (Duitburg) (2 cult.) . . .	A	‡ or A	A	A	—	A

AG=acid and gas, A=acid, ‡=variable or slow reaction; T=yellowish-white or grayish turbidity in media.
* No maltose fermenting strains were isolated in Kansas.

is stronger with *S. pullorum* than with *S. gallinarum*, and Klimmer and Haupt (1927) state that the reverse is true. Pacheco and Rodrigues studied the reactions on a variety of media with lead acetate, bismuth with and without cysteine, iron salts, and a peptone gelatin. *S. pullorum* produced H₂S rapidly on agar with lead and bismuth, both with and without cysteine, and slowly on the other media.

Hinshaw (1941) was able to separate the two species by use of a 0.15 per cent cysteine hydrochloride gelatin medium. *Salmonella gallinarum* in 89 of 91 strains produced a characteristic yellowish-white or grayish turbidity when incubated at 37° C. for 72 hours. *S. pullorum* did not produce such changes. Several maltose-fermenting variants of *S. pullorum* have been observed among those studied. None of these gave a positive reaction either in the cysteine-gelatin medium or in tartrate agar. He concludes that although many

variants exist, there is increasing evidence that these organisms are a distinct species.

Hinshaw reports that H₂S can be demonstrated readily in cultures of *S. gallinarum* growing in cysteine-gelatin by its faint odor and by use of strips of lead acetate paper. Only a faint browning of such paper is noted in *S. pullorum* cultures grown in this medium.

Table 10.2 contains a summary of the characteristics of variants of *S. pullorum* and *S. gallinarum*.

It is stated that there are probably several subspecies in the *pullorum-gallinarum* group. The Van Roekel maltose-fermenting strain gave reactions identical with those of the California maltose-fermenting strains. These strains differ from the *Salmonella intermedius* A-type strains in that the latter are xylose-negative and dulcite-positive. The *S. intermedius* B-types were sensitive to *S. pullorum* bacteriophage and were considered by Nobrega (1935) as

variants of the latter organism. Hinshaw (1941) found that *S. intermedius* A and B types reacted like *S. pullorum* in both cysteine-gelatin and tartrate agar. He states that the maltose-negative strain of *S. gallinarum* of Van Roekel was so classified on the ground that it produced a positive *S. gallinarum* reaction in cysteine-gelatin, acid in tartrate agar, and was dulcitol-positive.

The dulcitol-negative *S. gallinarum* of Kujumgiesff differs from that of Delpy and Rastegar's (1938) type B, in that the latter is dulcitol-positive and tartrate-negative.

The Duisburg strains of *S. gallinarum* are like *S. pullorum* in that they do not ferment tartrate agar and do not give any reaction in cysteine-gelatin. They resemble *S. gallinarum* in the type of growth on nutrient agar, and in being maltose- and dulcitol-positive. They are more nearly like the *S. intermedius* type B.

Johnson and Rettger (1942) studied the basal nutrition of *S. gallinarum* and *S. pullorum* with sixteen amino acids and thioglycolic acid. With the exception of two strains which required nicotinic acid or its amide, 43 strains of *S. pullorum* did not require any vitamins, while vitamin B_1 proved to be highly indispensable for the growth of 22 strains of *S. gallinarum*. Nearly all strains of *S. pullorum* required glucose while *S. gallinarum* did not. None of the strains of the former required the addition of CO_2, while several of the latter required an appreciable amount for growth. Leucine was the most important single amino acid; tryptophane was not required.

Although there are certain cultural and physiological differences between *S. gallinarum* and *S. pullorum*, their serological and antigenic characters are identical. Some European investigators are of the opinion that chronic *S. pullorum* infection and fowl typhoid are two forms of the same disease. This opinion is not generally held in the United States. Rettger and Koser (1917) concluded as early as 1917 that despite the several characteristics which these organisms have in common,

and particularly the serological reactions, the organisms constitute two distinct types, and each holds a specific relationship to the disease with which it has been associated in the past.

Beck and Eber, Beaudette, Hadley *et al.*, Hendrickson, Mulsow, May, Goodner, Rettger, Koser, Manninger, van Heelsbergen, and others have tried to separate the *S. gallinarum* and the *S. pullorum* on the basis of the agglutination reactions but have not succeeded. Even by applying the absorption method, the two organisms proved to be identical. Kauffmann (1930, 1931) states that *S. gallinarum* and *S. pullorum* strains contain the same O-antigen. This has been confirmed by Edwards (1939) in more recent studies. Many investigators consider that it is not improbable that *S. gallinarum* and *S. pullorum* represent two varieties of one microorganism. (See particular description under pullorum disease.)

Rodrigues and Pacheco (1936) were unable to detect any antigenic difference between *S. gallinarum* and the intermediate types. Although some appear to doubt that it is worthwhile to differentiate between the organism of fowl typhoid and pullorum disease, most American investigators agree that the organisms are somewhat different in their fermentation reactions and the pathological changes which they produce in infected birds. The agglutination test will not differentiate between carriers of either type of infection. Hinshaw (1941) was able to differentiate the two organisms by the use of a 0.15 per cent cysteine-hydrochloride gelatin medium first reported by Hinshaw and Rettger (1936). After incubation at a temperature that does not liquefy the gelatin, a turbid halo appeared around the individual colonies in shake cultures and along the line of inoculation in stab cultures. None of the 454 strains of *S. pullorum* consistently produced visible change in the medium, or at best a surface pellicle. Species of bacteria which were irregular in their reactions, but which gave mostly negative results were 14 strains of *E. typhi*

and 3 strains of *S. anatum*. A few cultures of Pseudomonas and Proteus from turkeys gave these reactions.

The Jordan-Harmon sodium-potassium-tartrate medium (1928) was a valuable supplementary medium to use with the cysteine-gelatin. The *S. gallinarum* strains consistently produced acid on this medium while *S. pullorum* produced no change.

Blaxland et al. (1956) made a study of the differential characteristics of 1,007 cultures of *Salmonella pullorum* and 618 cultures of *Salmonella gallinarum*, based on growth characteristics, biochemical reactions, and the absence of form variation in *S. gallinarum*. These differences are related to the distinctive epizootiology of acute outbreaks of pullorum diseases and fowl typhoid as occurring in the field.

It is considered that the results, taken in conjunction with the work of earlier investigators, provide conclusive evidence that *S. pullorum* and *S. gallinarum* are separate and distinct species.

Williams and Harris (1956) compared 41 strains of *Salmonella gallinarum* and 23 strains of *Salmonella pullorum* by the sedimentation test, using ammonium sulfate, and found that an ammonium sulfate concentration of 265 grams per liter completely cleared the supernatant fluids of most *S. gallinarum* suspensions but had considerably less effect on the turbidity of suspensions of standard cultures of *S. pullorum*. These observations suggest that there may exist minor antigenic differences between *S. gallinarum* and standard strains of *S. pullorum* that are not demonstrable by conventional serological methods.

Goret et al. (1956) discuss the similarities of *Salmonella gallinarum* and *Salmonella pullorum*. Usually the term "fowl typhoid" is used to describe a disease of adult chickens due to *Salmonella gallinarum*. "Pullorum disease" is used to describe a septicemia of embryos and chicks due to *Salmonella pullorum*. This distinction is erroneous. Septicemic conditions may be produced by either organism and chronic typhoid lesions are produced by gallinarum and pullorum types. When *S.*

gallinarum and *S. pullorum* are studied serologically, it is found that both have identical serological antigens. All strains contain the I, IX, and XII antigens; none of the strains contain XII₂ antigen. Both organisms show considerable variation in fermentative reactions—*S. gallinarum* may be grouped into seven distinct classes and *S. pullorum* into nine classes. Lysogenic studies show that both organisms are identical in their phage reactions. *Salmonella gallinarum* and *S. pullorum* should be considered as one species—*Salmonella gallinarum-pullorum*.

Ishii et al. (1958) examined 225 non-motile strains of Salmonella Group D isolated from chickens, and found that if *Salmonella pullorum* and *S. gallinarum* were considered as two distinct types, then the characteristics differentiating them seemed inconsistent and variable. The epizootiological distinction between the two organisms emphasized by earlier workers was not confirmed. It was further considered that too frequent occurrence of interrelated cultures threatened the significance dividing the organisms into two types.

It was also stated that it is against the rule of classification of enteric bacteria to divide the organisms into two types by biochemical methods. Therefore, it was recommended, as stated by Kauffmann, that the organisms be classified into a single type, "S. gallinarum-pullorum," and that the conventional classification be regarded as biotypes without special names.

Trabulsi and Edwards (1962) studied the biochemical characteristics of *Salmonella pullorum* and *Salmonella gallinarum*, as well as those of several aberrant cultures of Salmonella Group D. In addition to the tests usually used to differentiate *S. pullorum* and *S. gallinarum*, the cysteine-gelatin medium of Hinshaw and the ornithine decarboxylase test were found valuable in distinguishing the two types. It was concluded that *S. pullorum* and *S. gallinarum* constitute two distinct biochemical types which should not be

joined. The rare occurrence of aberrant strains which probably are neither *S. pullorum* nor *S. gallinarum* should not be permitted to confuse the problem.

Resistance. In general the resistance of this organism is about the same as that of the other members of the typhoid and paratyphoid groups.

The fowl typhoid organism is killed within 10 minutes at 60°C. It remains viable in the dark for 20 days in ordinary and in distilled water, but dies in 24 hours when exposed to sunlight. When dried on glass plates and kept in the dark, the organism retains its viability for 89 hours; under the action of direct sunlight it is killed in a few minutes. The organism is killed by phenol in a 1:1,000 dilution and by bichloride of mercury in a 1:20,000 dilution, potassium permanganate 1.0 per cent in 3 minutes, and 2.0 per cent formalin in 1 minute. Agar cultures rapidly lose their pathogenic character, although they retain their antigenic properties for some time. According to Altara, *S. gallinarum* in the virulent state can be demonstrated in the bone marrow of carcasses three months after chickens have died of fowl typhoid. No doubt under certain conditions it lives for much longer periods. Kaupp and Dearstyne (1924a) reported that although direct sunlight destroyed the organism in a short time, it remained viable for 20 days when stored in water in the dark.

In Moore's (1946b) national survey of fowl typhoid, 75 per cent of the states reporting were uncertain as to the persistence of the organism in the soil, and 25 per cent claimed that it persisted from year to year.

In the experiments of Hall *et al.* (1919a) there was little danger of starting an outbreak in susceptible birds when such birds were put into a fowl typhoid-contaminated pen one week or more after removal of all sick birds. It was evident that the causative organism did not survive long after leaving the bird's body.

The resistance of this organism to the action of bacteriophage is of some interest.

D'Herelle (1919, 1922) examined the excreta of fowls and tested the bacteriophage for virulence against eight strains of bacteria. Bacteriophage activity was demonstrated from all excreta studied; some samples showed marked activity for all cultures used. This investigator claimed that his experiments with bacteriophage confirmed his conclusion that the immunity to an infection is assured at a time when the body contains a bacteriophage virulent for that organism. Mallmann (1931a) criticizes these observations because of lack of controls. It was found that the bacteriophage from one organism was easily adapted to another by use of mixed cultures. Bacteriophage was of no value in treating chicks either naturally or artificially infected. Munné (1937) obtained several cultures of *S. gallinarum* and *S. pullorum*, all of which were equally susceptible to the action of bacteriophage.

In two experimental and one natural outbreak of fowl typhoid, Hall, MacDonald, and Legenhausen (1919b) saw no benefit from the administration of bacteriophage either in the drinking water or by subcutaneous experimental outbreak or by subcutaneous injection in the natural outbreak.

Orr and Moore (1953) tested *Salmonella gallinarum* for longevity under various conditions. In cloth in the dark at room temperature the organisms remained alive for 228 days. On plastic cover slips some *S. gallinarum* organisms were viable up to 93 days. Organisms in distilled water in diffused light at room temperature were viable up to the time the water evaporated –88 days. *S. gallinarum* retained viability up to 43 days when subjected to daily freezing and thawing. A liver naturally infected with *S. gallinarum* was divided, one-half being kept at 7°C., and the other half at –20°C. The organisms in the liver kept at 7°C. survived two weeks, while those in the portion of liver kept at –20°C. were still alive at 148 days, even though they were twice accidentally thawed.

Smith (1955a) found that the average survival time of *Salmonella gallinarum* in

feces from infected chickens was 10.9 days when kept in a range house and 2 days less in the open. Survival time was longer in naturally dried specimens than in those kept moist.

Pathogenicity. The pathogenicity of fowl typhoid cultures has proved decidedly variable in the hands of different investigators, probably for the reason that they used cultures varying widely in virulence. Like most pathogenic microorganisms *S. gallinarum* loses virulence rapidly on artificial media. Hence, cultures of *S. gallinarum* should be passaged serially in their natural host, the chicken, before testing the pathogenicity of the organism. The pathogenicity of such cultures is best maintained in the lyophilized or frozen state. With a *uniformly pathogenic culture, most commonly used routes of exposure of chickens prove fatal.* Kaupp and Dearstyne (1925) reported 16 deaths out of 40 chickens artificially infected; 15 became visibly sick, and 9 showed no symptoms. Similar results were reported at the Kansas Station, whereas others report from 25 to 90 per cent loss.

Palmer and Baker (1928) studied six natural outbreaks of fowl typhoid on Delaware farms. Virulent strains from these outbreaks killed not over 33.3 per cent of the test fowls, and the investigators concluded that 60 to 70 per cent of fowls are naturally immune. Hall, Legenhausen, and MacDonald found that feeding mash moistened with a broth culture of a stable virulent strain of *S. gallinarum* was an effective way of testing susceptibility to fowl typhoid. Of 20 groups of birds, aggregating 382, which were challenged by adding a broth culture of virulent strains of *S. gallinarum* to their mash, 367, or 96 per cent, died of fowl typhoid.

Although fowl typhoid frequently has been spoken of as a disease of adult birds, Beaudette (1925), Beach and Davis (1927), Martinaglia (1929), and Komarov (1932) reported the disease in young chicks. Moore (1946b), in a nationwide survey of fowl typhoid, reported that 11 states found the

disease to be more common in birds under six months, 16 believed the disease to be more prevalent in older fowl, while 10 states reported that there was little difference in age susceptibility. Hall *et al.* (1949a) reported that in 25 hatches from typhoid reactors in their second year of lay, about every fourth hatch experienced a fowl typhoid outbreak and losses up to six months of age amounted to 33.4 per cent of the chicks hatched. Monthly distribution of mortality was as follows:

Month	Per Cent
1	25.6
2	13.5
3	24.9
4	19.2
5	13.8
6	2.7

As in pullorum disease, fowl typhoid losses often begin at hatching time; but contrary to experience with pullorum disease, fowl typhoid losses continued through to laying age. In one experiment in which two lots of chicks were hatched from fowl typhoid reactors, 92.8 per cent of the chicks hatched in one lot died within 16 days and in another lot 93.5 per cent died within 11 days after hatching.

Epizootiologically there are a few peculiarities in regard to the disease. Van Heelsbergen (1929) states that according to his experience it is very difficult, in some cases at least, to infect chickens which come from a region to which fowl typhoid is indigenous. If chickens are imported from a part of the country where the disease is not known, infection is rather easy. It is suggested that the bacteriophage, or acquired immunity, is probably in part responsible.

St. Johns-Brooks and Rhodes (1923) found that strains of *S. gallinarum* produced lesions in young chicks indistinguishable from those associated with pullorum disease.

A relatively small number of avian species appear to be susceptible. Lucet (1891) described what was probably an

outbreak of the disease in turkeys but claimed that ducks, geese, and pigeons were not susceptible. Donatien et al. (1923) consider palmipeds to be refractory, but found the turkey, guinea fowl, and peafowl among the susceptible species; ducks and geese were resistant. Pfeiler and Roepke (1917) mention the pheasant, turkey, and guinea fowl as susceptible in natural outbreaks, but that ducks, geese, and pigeons are not, although a duck which had been inoculated with a culture died a few days later. Kaupp and Dearstyne (1924), Beck and Eber (1929), and te Hennepe (1924) have observed the disease in ducks. Kaupp and Dearstyne (1925) state that turkeys are less susceptible than chickens, and that guineas, though slightly susceptible, yield to artificial inoculation. Fox (1923) isolated B. sanguinarium from an outbreak of disease among parrots in The Philadelphia Zoological Garden. Beck and Eber (1929) reported on the loss in ducklings 1 to 14 days old due to B. gallinarum infection. Truche (1923) found that pheasants, swans, grouse, sparrows, ring doves, and ostriches commonly became infected, but that the duck, goose, and turkey were more resistant. Johnson and Anderson (1933) reported outbreaks of the disease in ducklings, turkeys, and guinea fowl. The infection has been observed in wild birds, in quail, grouse, and pheasants. These birds are susceptible by feeding or injection of cultures. Te Hennepe (1939) states that fowl typhoid has decreased in the Netherlands during the past ten years from a point at which it caused some 8.0 per cent of the total deaths in adult birds to 0.7 per cent in 1939. This is considered to be due to greater interest in poultry diseases and improved care of poultry. The disease was at one time one of the most important in Kansas. About 1935 it practically disappeared and is still quite uncommon. The reason for this is not known. El-Dine (1939), in Egypt, states that fowl typhoid is often mistaken for fowl cholera. He reports the disease mainly in chickens and

turkeys, and states that it has been reported in peacocks but has never been seen in pigeons, geese, or ducks. A vaccine was used to confer immunity.

The reports on the susceptibility of pigeons have been variable. Klein reported no success following subcutaneous injection of cultures. Lucet (1891) was unable to infect pigeons with 1.0 cc. doses subcutaneously, while Moore (1895) killed pigeons within 8 days with 2.0 cc. of a broth culture. Pfeiler and Roepke (1917) killed pigeons by injecting 1.0 cc. of a 24-hour broth culture, but the heart blood of these birds would not cause infection in a second pigeon. Kaupp and Dearstyne (1924) caused the pigeon to become sick on the third or fourth day with recovery on the fifteenth day. Kraus (1918) produced death in a pigeon within 4 days by use of 1.0 cc. of a 24-hour broth culture of the fowl typhoid organism. Te Hennepe and van Straaten (1921) claim that pigeons are not always susceptible to inoculation with these organisms. At the Animal Disease Station in 1946, four pigeons were killed in an average period of 4.5 days by intraperitoneal or intramuscular inoculation with 1.0 ml. of a 5-hour broth culture of S. gallinarum.

Hinshaw (1930) reported that the disease caused greater losses among California turkeys than did blackhead. Hinshaw and Taylor (1933) reported an ovarian infection of a turkey hen from which they isolated the organism. Hudson and Beaudette (1929) state that there is a difference of opinion as to the incidence of fowl typhoid among avian species, but that there is evidence that it is greater than is ordinarily suspected. A lack of accurate field diagnosis undoubtedly results in many discrepancies. Lerche (1939) reports one case of an infection of humans with a culture very similar, but not identical, to S. gallinarum, and the Duisburg strain of S. gallinarum was originally obtained from linarum was originally obtained from acute gastroenteritis in man. Cloud (1943) reported the isolation of the Duisburg strain from a patient with severe

peritonitis. The organism was not found in the stools. However, this organism should not be considered a human pathogen.

It is not difficult to infect rabbits with fowl typhoid bacilli. Pfeiler (1920) succeeded in inducing infection four times in 15 rabbits. Guinea pigs and pigeons are very resistant, although, as has been observed repeatedly, pigeons die if the dosage is somewhat large. Van Straaten, te Hennepe, and Pfeiler were quite successful in infecting white and gray mice, while rats, dogs, and cats were shown to be immune. A relatively small number of avian species appear to be susceptible, although there is a difference of opinion as to the incidence of this disease. Hinshaw and Taylor (1933) inoculated two mature rabbits intravenously with 0.5 and 1.0 cc., respectively, of a 48-hour broth culture; the animals lived. The one receiving 1.0 cc. was killed three weeks later, and the blood was found infectious for young rabbits. Smith and Ten Broeck (1915) stated that the pathogenicity of *S. gallinarum* was relatively feeble for laboratory animals. The rabbit succumbed to relatively large doses (0.3–0.5 cc.) of a 24-hour broth culture given intravenously. Pfeiler and Rehse (1913) state that pigeons, geese, and ducks are resistant to the infection, but mice succumb. Rats, cats, and dogs fail to show any disturbance after eating diseased material.

Smith and Ten Broeck found a toxin in the filtrates of broth cultures of *S. gallinarum*. It appeared in the culture at the end of 2 days at 37° C. and caused prompt death of a rabbit by the intravenous route. Death resulted within 2 hours and in many respects was like an anaphylactic shock. It is probably an endotoxin which is stable at 60° C. for 1 hour. Boiling for 15 minutes reduces its activity.

Rao et al. (1952) reported *Salmonella gallinarum* to be equally pathogenic to baby chicks and adults under natural conditions.

They all reported their organism to be fatal to pigeons, guinea pigs, and rabbits.

Smith (1955b) reported that immunity appeared to be solid when massive doses of *Salmonella gallinarum* were administered either orally or intravenously to clinically recovered chickens.

One-day-old chickens were most susceptible. This was followed by a decrease in susceptibility until maturity when it again increased. There was no difference in susceptibility due to sex.

Van Es and Olney (1940) exposed fowls to artificial infection and observed that the losses were variable and that the birds which contracted the disease showed a considerable degree of variation in the length of their survival. This difference was probably due to individual variation in susceptibility.

Symptoms. The incubation period, as a rule, is from 4 to 5 days, although this varies considerably with the virulence of the organism, and the course of the disease is about 5 days.

Comb and wattles are generally pale and shrunken, especially in the subacute cases. In acute cases, however, the comb and wattles may be dark colored, as in fowl cholera. The birds become listless and inactive and prefer to separate from the flock. A thin greenish-yellow diarrhea appears early; there is complete loss of appetite; intense thirst as in fowl cholera is common, presumably as the result of high fever. Investigations at the Kansas Station would indicate that there is no difference in the temperature range of fowl cholera and fowl typhoid; temperatures of 110° to 112° F. are common. Respiration is at first accelerated. In some cases death occurs suddenly at the end of the second day. Comb and wattles appear anemic instead of cyanotic as in fowl cholera. The anemic condition becomes pronounced in prolonged cases. Mortality is variable. In acute outbreaks losses may be heavy in the beginning, followed by a tapering off to an occasional death in the chronic stage. Fowls visibly sick generally do not survive.

Pathology. In peracute cases little or no gross tissue changes are observed. In the more prolonged cases, however, marked

FIG. 10.1 — Subacute fowl typhoid. Grayish-white foci in myocardium and swollen "bronze" liver.

changes begin to appear, the most common of which are swelling and redness of the liver, spleen, and kidneys. These lesions are frequently seen in young birds. In the subacute and chronic stages of the disease the greenish-brown or bronze and swollen livers are commonly seen. Other changes include grayish-white foci of the miliary type in the liver and myocardium, pericarditis, peritonitis due to ruptured ova, hemorrhagic, misshapen and discolored ova, and catarrhal inflammation of the intestines. In young chicks grayish-white foci may sometimes be observed in the lungs, heart, and gizzard, as in pullorum disease (Fig. 10.1).

In acute cases the blood picture is changed very little. If the disease lasts a few days, however, the changes mentioned in Table 10.3 are observed.

Beck and Eber (1929) recognized great loss from *B. gallinarum* infection among ducklings 1 to 14 days old. Maltose, dulcite, and dextrin were fermented with acid formation by the organism isolated. The disease picture was similar to the one observed in pullorum infection in chicks. The ducklings were sick only a short time. The anatomical changes were the following: hemorrhage in the pericardium, slight swelling of the spleen, catarrhal inflammation of the lungs and intestines. Small necrotic foci in the lungs, as frequently observed in chicks with pullorum disease, did not occur. In adult ducks the changes of the ovary and the yolk were frequently the same as those found in adult hens. Fowl typhoid organisms were isolated from the misshapen yolks.

Gauger (1934) obtained the organism of

TABLE 10.3

CHANGES OF THE BLOOD WITH FOWL TYPHOID (WARD AND GALLAGHER, 1920)

Date	Temperature in C.	Number of Red Blood Corpuscles Per cm.	Number of White Blood Corpuscles Per cm.	
March 26 ...	41.5	3,535,000	18,940	Healthy
March 28 ...	43.5	2,430,000	70,000	Chicken eats very little
April 2 ...	43.8	1,684,210	80,000	Blood very pale; chicken weak, refuses food
April 3 ...	41 3	1,745,000	245,000	Very weak, very many red blood corpuscles attacked by leucocytes
April 4 ...	......	......	......	Found dead

fowl typhoid from focal lesions in the testicles of a rooster. The culture was pathogenic for other roosters by inoculation and feeding. Although various foci had been described, this was the first case described for focalization in the testicles.

Beaudette (1938) states that the disease in the guinea is interesting because the affected birds show respiratory symptoms characterized by a severe congestion with collection of mucus in the nasal cleft and trachea. The lungs were congested, and the organism could be isolated from the nasal exudate.

Johnson and Pollard (1940) studied an outbreak of disease which resembled pullorum disease in week-old turkey poults. The necropsy revealed a large retained yolk, and the liver appeared somewhat friable and of creamy-white color. The surface was mottled with slight hemorrhagic areas. A slight congestion of the anterior portion of the duodenum was found. The organism isolated was a Gram-negative rod producing acid but no gas from dextrose, mannite, dulcite, xylose, sorbite, arabinose, maltose, levulose, and dextrin, but did not react with lactose, sucrose, or inositol. Serologically the organisms checked with S. gallinarum. This organism was isolated from the ovaries of the adult birds that supplied the poults. It appeared to be a very chronic disease in these birds. By testing at frequent intervals with a pullorum antigen the percentage of reactors in the flock was reduced from 8.7 to 6.0 per cent. However, it was considered as doubtful if the infection could be completely eliminated in this manner.

Rao et al. (1952) reported the presence of a band of hemorrhage in the submucosa of the proventriculus, and cyanotic instead of anemic comb and wattles in the adult.

Smith (1955b) produced fowl typhoid in chickens by the oral administration of Salmonella gallinarum. Infection took place in the intestinal tract with localization in the intestinal wall, liver, and spleen. This was followed by a bacteremia and death or chronic disease with proliferative lesions in the intestinal and heart walls. Of 300 nine-week-old cockerels, 45 per cent died of the acute disease and 15 per cent died in the chronic stage. S. gallinarum was shed in the feces up to two to three months after infection. This was associated with focal infection in the intestinal wall.

Diagnosis. In general it is not difficult to diagnose fowl typhoid clinically. The disease is not as acute as fowl cholera, monocytosis (pullet disease), or fowl plague. In these latter diseases, as many birds are sometimes lost in a few hours as are lost with fowl typhoid in as many days. On necropsy the differences exhibited by these diseases are generally quite evident. The marked swelling of the liver in fowl typhoid is not found in fowl cholera and fowl plague, and the general hemorrhagic character of the last two diseases is more

TABLE 10.4
FERMENTATION REACTIONS OF COMMON POULTRY PATHOGENS

	Dextrose	Lactose	Maltose	Sucrose	H₂S	Indol
S. gallinarum	AG	—	?	—	?	—
S. pullorum	AG	—	— *	—	+	—
S. anatis	AG	—	AG	—	+	—
S. typhi-murium	AG	—	AG	—	+	+
P. avicida	A	—	—	A	—	+
E. coli	AG	AG	AG	?	?	+
Staphylococci	A	A	A	A	—	—

AG = acid and gas; A = acid; — = no change; ? = slight; + = positive.
* Of the large number of cultures of S. pullorum which were isolated in the Laboratory of Bacteriology, Manhattan, Kansas, the maltose-fermenting type was very rare.

common. Petechiae are observed in fowl cholera or fowl plague more frequently than in fowl typhoid. The bronze-colored liver makes almost certain the diagnosis of fowl typhoid. Also, the microscopic blood picture differs in fowl typhoid from that in fowl cholera and fowl plague. A few rods resembling colon organisms may be seen in the preparations from cases of fowl typhoid; in fowl cholera the bipolar organisms are very common; in plague no bacilli are found in the blood. As a means of differential diagnosis a chicken and a rabbit may be inoculated with some material from the diseased bird. In fowl cholera both animals die in 1 or 2 days; in plague, only the chicken dies in 2 to 4 days; in fowl typhoid, the rabbit generally survives, the chicken remains alive or dies after 6 to 10 days.

A laboratory diagnosis is quite easy. The organism grows readily on ordinary laboratory media and may be identified by bacteriological or serological methods. Table 10.4 will be of value in this diagnosis.

The S. anatis may be separated from S. typhi-murium by fermentation of inosinol. S. anatis fails to ferment it while the latter ferments it with production of acid and gas.

A positive antipullorum serum will be of value in making a slide agglutination test. This, however, will not differentiate the two organisms, but is a valuable adjunct to the fermentation reactions.

Mallmann et al. (1928) reported on the use of brilliant green in the isolation of S. gallinarum. The growth of the organism was not affected by freshly prepared solutions of 1 to 75,000, while the dye exerted an inhibitory effect on E. coli, as previously described by others. The bacteriostatic effects of different lots of dye from different manufacturers were found to be decidedly different. These results were confirmed by Kerr (1950). Delpy and Rastegar (1938) found that this dye could be used to advantage for the isolation of the pullorum-gallinarum group and its intermediates.

Fowl typhoid in chickens may be confused with fowl cholera and monocytosis in the field. This is especially true if the diseases are acute. If the birds die quickly with fowl typhoid, the liver is lighter than normal, with pale streaks. In monocytosis the most characteristic change of the liver consists of round, yellowish areas with hemorrhagic centers. No degeneration of the skeletal muscle is observed in fowl typhoid, while it is fairly diagnostic for monocytosis.

It should be emphasized that the final differential diagnosis must be made in the laboratory where the organism can be isolated and identified. (No organism can be isolated which will cause monocytosis.)

At the twenty-second annual meeting of the Northeast Conference of Laboratory Workers in Pullorum Disease Control (1950), the committee on standardization

of cultural examination of reactors for fowl typhoid recommended the use of an enrichment medium such as selenite. Into 20 cc. of this enrichment broth a one gram portion of the organ to be cultured is dropped. After 24 hours incubation the culture is plated on SS agar and bismuth sulfite agar. The use of tetrathionate broth, with brilliant green as an enrichment medium and MacConkey's agar, desoxycholate, and D.C.L.S. as a selective medium, is optional. Identification is made by the use of dextrose, lactose, sucrose, and maltose, the first and last of which are fermented. Reactors are further identified by the indol test and urease activity.

The agglutination test, using antigen made from S. gallinarum or S. pullorum, can be used to detect the birds infected with S. gallinarum.

METHODS OF CONTROL

In the control of infectious diseases of poultry, several procedures are available, including prevention (immunization, sanitation, and breeding for resistance), treatment, and eradication (elimination of carriers and depopulation). The procedure to employ depends on the type of disease being dealt with and the type of poultry industry in which it is found. Most well-established virus diseases are best controlled through vaccination. On the other hand, most bacterial diseases respond poorly to vaccination, but some of them can be controlled by detection and elimination of carriers. The latter procedure is highly recommended for the control of fowl typhoid.

Van Roekel (1962) discusses additional measures required for the eradication of pullorum-typhoid infections, and cites the following procedures which were adopted and recommended in 1960 by the American Association of Avian Pathologists as a guide for a basic eradication program:

1. The basic principles for identification of a U.S. pullorum-typhoid-clean flock under the National Plans are acceptable as the starting point for an eradication program.

2. A federal regulation should be adopted to control the interstate movement of poultry from the standpoint of pullorum disease and fowl typhoid. If this does not receive adequate support, then each state should develop its own state regulation controlling the importation of hatching eggs and poultry from the standpoint of pullorum disease and fowl typhoid.

3. All turkey and chicken breeding flocks as well as other fowl in the state should be under a pullorum-typhoid control program and should be classified as U.S. pullorum-typhoid-clean or the equivalent.

4. All outbreaks of pullorum disease and fowl typhoid should be reported to the proper state agency with regulatory power, such flocks should be quarantined, and the marketing of such flocks should be in a plant under state or federal supervision. Other types of Salmonella and Arizona infections should be reported to the proper state agency.

5. Poultry consigned to public exhibitions such as county and state fairs and poultry shows should originate from U.S. pullorum-typhoid-clean flocks or the equivalent.

6. A state or area may be designated as pullorum-typhoid-free if all turkey and chicken breeding flocks are classified as U.S. pullorum-typhoid-clean or the equivalent and no flocks are under quarantine in the area.

Wilson (1958) reports an increased incidence and wider distribution of fowl typhoid in recent years. Control measures consist of prompt diagnosis, immediate therapeutic treatment with furazolidone 0.04 per cent for 10–12 days, and removal to fresh quarters preferably followed by another course of treatment and the use of the agglutination test to detect carriers. An effective preventive vaccine is not at present available, but one now under test has shown promise.

Machan (1959) from twenty years' ex-

perience with slow serum and rapid whole-blood agglutination tests for pullorum disease stated that the disease cannot be efficiently eradicated from an infected flock by systematic blood testing at 6-week intervals unless the houses and yards are thoroughly disinfected. In Austria, delivery of hatching eggs, permitted according to official regulations, for 9 months from the time of blood examination, has proved too long a time in practice. The reduction of this period to 6 to 8 weeks is recommended by the appropriate ministry for infected flocks.

Winmill (1961) recommends the following procedure for the control of fowl typhoid: (a) destruction of all sick birds; (b) complete destocking of all infected pens; (c) vaccination with 1909S of all birds separated from the focus of infection and negative to the agglutination test; (d) repeated vaccination of young birds (at 8 and 16 weeks of age).

Breeders who vaccinate laying fowls with 1909S are advised to avoid the incubation of eggs for six weeks after vaccination.

The control of *Salmonella pullorum* infection (pullorum disease) by agglutination testing and removal of reactors was begun on a nationwide scale in 1936 through the adoption by the poultry industry on a voluntary basis of the National Poultry Improvement Plan (now the National Poultry and Turkey Improvement Plans) suggested by the Poultry Research Branch, Animal Husbandry Division, Agricultural Research Service, U.S. Department of Agriculture, but it was not until 1951 that the closely related *Salmonella gallinarum* infection (fowl typhoid) was included in the Plans. The disease control section of the national Plans through its agglutination testing and removal of reactors has been very successful in reducing pullorum disease to a small fraction of 1 per cent of the birds tested. It is hoped this testing program will reduce the incidence of fowl typhoid as well, and that it will also prevent the vastly

destructive fowl typhoid epizootics such as that which swept the eastern seaboard in the 1940's including "pullorum-clean" flocks.

Prevention

1. Immunization. Attempts have been made to immunize fowl with killed fowl typhoid cultures. According to some writers the results of this type of treatment are of little value. However, it cannot be denied that killed bacteria of the group possess some antigenic properties. McNutt (1926) experimented with various commercial vaccines on about 725 chickens and concluded that they have no value for the control of fowl typhoid. It is probable that repeated vaccination will produce a better degree of immunity than one treatment. Experiences from field practice point in that direction. Bushnell and Patton (1924) found that three vaccinations at about 5-day intervals reduced the mortality from some 30 to 5.6 per cent in birds occupying contaminated runs. The results of vaccination are difficult to evaluate because of the natural variation in normal resistance.

Panisset (1950) states that vaccination against fowl typhoid, in spite of its imperfections and its failures, even when carried out with stock vaccines is in the majority of cases an effective weapon in the struggle against the disease. Strict attention must be paid to the observation of hygienic measures. Referring to the control of both fowl typhoid and pullorum diseases, Manninger (1950) states in part that no results whatever toward the control of the disease can be obtained by the use of biological products. However, an efficient method of control consists in careful elimination and killing of the reactors. The agglutination test renders quite satisfactory results, and a prompt and reliable eradication of the disease from a flock depends largely on repeated blood testing. Dearstyne *et al.* (1933) recommended that control practices revolve around a rigid quarantine of the flock; destruction

of all birds showing symptoms of the disease; disinfection of houses, utensils, and drinking water; and the vaccination of all well birds.

Coles (1946) recommends for prevention two vaccinations a week apart, each consisting of a 1 cc. subcutaneous injection. Immunity takes 12 to 14 days to develop and lasts about 9 months. He states that successful eradication of fowl typhoid depends on a combination of:

1. Hygiene, i.e., clean houses, uncontaminated food and water, and proper disposal of feces.
2. Vaccination — failures of vaccination are said to be due to neglect of the necessary hygienic measures.

Wilson (1946) found killed culture vaccines ineffective in protecting against artificial exposure to S. gallinarum. This was true of both autogenous and stock vaccines. However, when a dose of autogenous vaccine was followed by a dose of live culture, a solid and lasting immunity was produced, but this procedure was not recommended.

Hall et al. (1949b) reported experiments on the use of bacterial vaccines in fowl typhoid. Vaccines were prepared by killing cultures of S. gallinarum with formalin, phenol, chloroform, brilliant green, and crystal violet. All except the crystal violet vaccines were suspended in beeswax and peanut oil. Injections were made subcutaneously, intraperitoneally, intramuscularly, and into the crop. No significant protection was observed after the vaccinated birds had been exposed to infection two to four weeks later.

Smith (1956) reports that a good immunity was produced in chickens against oral infection with Salmonella gallinarum by the use of either a smooth (9S) or rough (9R) attenuated vaccine. Killed vaccines had no effect. The immunity produced by the rough strain was limited to about 12 weeks, while that produced by the smooth strain was complete to at least 34 weeks.

The rough strain vaccine, 9R, did not produce agglutinins against smooth S. gallinarum in chickens. It was not lethal to one-day-old chicks, and did not cause a fall in egg production in laying hens. On the other hand, the smooth strain, 9S, produced agglutinins, killed one-day-old chicks, and was accompanied by a marked drop in egg production.

Gordon et al. (1959) submit evidence confirming the efficiency of attenuated live vaccines of Salmonella gallinarum 9S and 9R in conferring immunity against fowl typhoid.

There was no difference in susceptibility to, nor in response to, either vaccine among the four breeds tested.

Vaccination at 8 weeks of age produces an appreciably better immunity than at 4 weeks of age.

The use of vaccine 9R in the field appears justified in that it confers an appreciable degree of immunity without seriously interfering with the routine blood test for carriers of Salmonella pullorum and, in addition to possessing a low level of virulence for chicks, its transmission through the egg from vaccinated laying birds appears to be almost negligible.

The avirulent smooth strain 9S provided the better immunity but has the disadvantage of interfering with the blood test.

Gordon and Luke (1959) confirmed the value of the 9R vaccine in the control of fowl typhoid in Northern Ireland. In two breeding flocks blood agglutinins developed to the extent of 6.12 per cent and 5.83 per cent respectively.

The vaccinal strain was isolated 11 months after vaccination and there was presumptive evidence that vaccination of adult birds with the rough strain may induce pathological changes in the ovary in some birds.

Harbourne (1957) conducted field trials to assess the value of two live attenuated vaccines, one a rough strain of Salmonella gallinarum (9R) and one a smooth strain (9S) as a means of preventing and controlling fowl typhoid.

Preventive vaccinations were performed

at 43 farms; one-third of the birds of each flock received one ml. of vaccine 9R subcutaneously, one-third 0.2 ml. of vaccine 9S subcutaneously, and the remaining one-third was left unvaccinated.

In 18 flocks where losses from fowl typhoid were slight, 0.9 per cent of the deaths occurred in birds vaccinated with 9R, 0.4 per cent in birds vaccinated with 9S, and 2.4 per cent in unvaccinated controls.

At seven of the farms where losses from fowl typhoid were appreciable, after the first 14 days from the start of the trial 1 per cent of the birds vaccinated with 9R, 1.7 per cent vaccinated with 9S, and 11 per cent of the unvaccinated birds died from fowl typhoid.

At two of the farms the survivors were killed without blood testing. A smooth strain of *S. gallinarum* was recovered from a high proportion of the birds in each of the 3 groups.

In five flocks where fowl typhoid had previously been diagnosed, two-thirds of the birds of each flock received one ml. of 9R vaccine subcutaneously; the remaining third was left unvaccinated. Significant losses from fowl typhoid occurred at two farms. At one of these farms vaccination apparently precipitated death. At the other, 5 per cent of the vaccinated birds and 39 per cent of the unvaccinated birds died from fowl typhoid.

It is suggested that treatment with furazolidone followed by vaccination might be a useful procedure in flocks where an early diagnosis of fowl typhoid is made.

2. Sanitation. In the prevention of the spread of infectious diseases, sanitation appears to play an important role. Van Es and Olney (1940) subjected two groups of birds to opposite extremes in sanitation, and noted the effect on losses from fowl typhoid. In the sanitary yard the ground was covered with gravel and the house had wire floors. The water fountains were self-cleaning, and the feeders were covered to prevent contamination as much as possible. In the other pen the yard was dirt and ungraded, and the birds ate and drank from open vessels. A total of 80 fowls succumbed to fowl typhoid from the two pens when infected fowls were introduced. In the sanitary pen 10 died from fowl typhoid, and 70 died in the unsanitary pen.

On the other hand, Hall *et al.* (1949a) found that an unsanitary pen (one highly contaminated with the discharges of fowls sick with acute fowl typhoid) produced only 20 per cent as much fowl typhoid in susceptible fowls as the same pen occupied by sick birds when the susceptible birds were put in. In other words, these experiments indicate: (1) that the sick bird is probably the worst spreader of the disease and should be removed promptly, and (2) that the fowl typhoid organism, *Salmonella gallinarum*, rapidly loses virulence after discharge from the bird's body, and consequently, a short period of vacancy before restocking greatly reduces the probability of an outbreak.

After an outbreak the following sanitary measures may be helpful: Very sick birds should be killed and burned or buried deeply. The building must be thoroughly cleaned; walls, perches, nests, and apparatus should be scrubbed with lye water (1.0 pound of lye dissolved in 15 gallons of hot water). The upper surface of earth floors should be replaced with fresh soil; cement floors should be cleaned and disinfected. The dropping boards should be cleaned. Feeding pans and drinking vessels as well as all other utensils should be thoroughly scoured with lye water and rinsed. Sometimes a hot solution is preferable because of the resistance of protozoan parasites to the action of chemical disinfectants. This is especially true in case of coccidia and worm eggs. After the building has been cleaned, dried, and aired, the walls may be whitewashed. An antiseptic whitewash may be prepared by adding 5.0 per cent crude carbolic acid. Yards and runs should be arranged so that all parts are exposed to direct sunlight at some time during the day.

Puddles and pools must be eliminated. Pigeons, mice, and rats are to be kept away from the premises. If there are several chicken houses to be served by the same attendant, a mat dipped in disinfectant may be placed so that shoes are cleaned before entering each house.

Botts *et al.* (1952), investigating the bactericidal effect of old built-up litter compared with new cob litter, found that when six-week-old chicks were placed on the litters 48 hours after they were sprayed with *S. gallinarum*, the mortality was lower on old built-up litter than on new cob litter.

3. Increasing resistance by breeding and by diet. Breeding and selection for resistant strains of birds may be an important means of control. Lambert (1933) showed that selection for resistance to fowl typhoid in chickens resulted in a decided decrease in the mortality of selected stocks. Since *S. gallinarum* and *S. pullorum* are closely related organisms, it was decided to test fowl typhoid resistant stock for susceptibility to *S. pullorum* infection, and an *S. pullorum* stock for susceptibility to *S. gallinarum*. Results indicated that selection for resistance to one pathogen affords some protection to infection with one closely related. It was suggested that the resistance was to some extent due to nonspecific factors.

Garren and Hill (1959) made agglutinating antibody determinations for White Leghorns, Rhode Island Reds, and White Leghorn-Rhode Island Red crosses after inoculation with live and killed *Salmonella gallinarum* cultures (fowl typhoid). White Leghorns consistently developed lower antibody titers than Rhode Island Reds whether induced by infection or by bacterin. Leghorn-Red crosses were intermediate between the two breeds in antibody titers but possessed almost the same marked resistance to fowl typhoid as observed for the Leghorn.

Smith (1954) found that different infection rates were observed when different foods containing similar numbers of *Salmonella gallinarum* were fed to chickens.

The main reason for this was that certain properties of some of the foods had a profound influence on the bactericidal action of the gastric juice in the gizzard. One of these properties was physical consistency, and another was the ability to maintain a relatively high pH in the gizzard. A diet of whole wheat only was especially effective in lowering the pH of the gastric juice with consequent destruction of *S. gallinarum* and a decrease in the infection rate. It is suggested that when a natural outbreak is diagnosed in a flock it would be worthwhile to alter the diet to whole wheat only until it is possible to institute other methods of control.

Hill and Garren (1955) demonstrated that high levels of all known required vitamins increase the resistance of chicks to fowl typhoid. The enhanced resistance observed when high levels of vitamins are fed is apparently not due to a uniform increase in requirements for all vitamins. Some vitamins must be increased over the requirement for growth many times more than others in order to bring about increased resistance to typhoid. In addition to the essential vitamins at high levels, an antioxidant is required in the diet in order to increase resistance of the chick to fowl typhoid. It was also found that it is possible to oversupplement the diet with vitamins insofar as obtaining maximum resistance to oral inoculation of the *Salmonella* organism. The exact mechanism of all the phenomena observed in these studies is not known.

Hill and Garren (1958) showed that the plasma ascorbic acid levels of chicks with fowl typhoid were reduced. Supplementation of the diet with 0.1 per cent ascorbic acid resulted in an increased plasma ascorbic acid level at all times throughout the experimental period.

From the sixth to the ninth day, the period of heaviest mortality, the plasma ascorbic acid level of the basal-fed group was unchanged, while that of the supplemented group increased significantly. During this time, 20 per cent of the basal-

fed group died, while only 11.2 per cent of the supplemented group died. This difference was statistically significant ($X^2 = 4.620$, P .05).

Chubb et al. (1958) testing the effect of feeding high levels of vitamins on the susceptibility of 3 breeds of chickens to experimental Salmonella gallinarum infection found that the administration of high levels of all the required vitamins in the diet for four weeks prior to infection or at infection had no effect on the average survival time or total mortality in 8-week-old Rhode Island Red or Brown Leghorn chickens. Neither did separation of these vitamins into the fat-soluble or water-soluble groups and their administration in the diet of high levels for four weeks prior to infection have any effect on the average survival time or total mortality in these two breeds of chickens.

With White Leghorn chickens infected with Salmonella gallinarum a great variation in response to the high level feeding of vitamins was encountered. Some results would suggest that the feeding of high levels of vitamins for four weeks prior to infection may increase the susceptibility of this breed to Salmonella gallinarum and this may possibly be associated with the fat-soluble group (A, D, E, and K).

Smith and Chubb (1957) found that the protein level of the diet affected the mortality rate of chickens infected with Salmonella gallinarum. A simple diet of ground wheat plus 2½ per cent fish meal gave the highest resistance to S. gallinarum infection. A much higher or lower level of fish meal had an adverse effect on resistance to infection.

Starvation for 48 hours increased the severity of the disease.

Hill and Garren (1961) found that increasing the protein level of diets from 10 per cent to 20 to 30 per cent resulted in a progressively increased rate of mortality of chicks from Salmonella gallinarum infection. The rate of mortality was not affected by the energy content of the diet. The acceleration of mortality was evident whether the protein was supplied by soybean meal or casein. Since the acceleration was noted when the organism was given either orally or intramuscularly, it was concluded that the effect of the increased protein level did not depend on a meeting of pathogen and diet in the intestinal tract. While total mortality was generally increased with increasing protein levels, the differences were not statistically significant in most of the comparisons.

Treatment

Sulfonamide drugs have been tried by numerous investigators with conflicting results. Hammond (1945) reported effective control by use of sulfathiazole. Holtman and Fisher (1946) studied an outbreak in battery-raised chickens which had caused 20 per cent loss in 3 days. The flock was then divided into two parts. Group 1 received the usual care — removal of sick birds and cleaning and disinfection of batteries with a cresol solution. Group 2 was given sodium sulfathiazole in the drinking water for one week. At the end of the week 80 per cent of the birds in group 1 had died. Losses in group 2 had been reduced to 4 per cent with no losses during the last 3 days. However, losses in this group reappeared within 5 days but were controlled by the use of the drug.

The next year, Holtman and Fisher (1947) were successful in controlling natural outbreaks of fowl typhoid in broilers by the use of sodium sulfamerazine (0.2 per cent) in the drinking water. There was a loss of 62 per cent in the controls compared to 4 per cent in the treated birds when the drug was administered for 5 consecutive days each month for 2 months.

Simms (1946) reported on the use of sulfamerazine, sulfadiazine, and sodium sulfathiazole in broiler plants. The drugs were fed in 0.5 to 1.0 per cent in wet mash. None of these drugs was satisfactory for controlling a virulent outbreak of the disease. Mortality was greatly reduced while the drugs were being fed, particularly in the case of sulfamerazine, but on discon-

tinuance of its use, mortality rose again to nearly its former level. The same results were obtained from the use of these drugs in breeding flocks.

Moore (1946c) reported that sulfamerazine was effective in reducing death losses from fowl typhoid, while sulfathalidine and sulfasuxidine were not effective. The mortality varied from 33.3 to 83.3 per cent for these two drugs. However, the former was given 5 days after exposure while the latter was started at the time of exposure.

Alberts (1950) used 0.4 per cent sulfamerazine-mash mixture, or 0.2 per cent sodium sulfamerazine in the drinking water during a 7-day course of treatment. This prevented losses during the 7-day period of treatment and for 2 days after. The intermittent use of sulfamerazine or sodium sulfamerazine over a 21-day period was more effective in minimizing losses than was continuous feeding of the drug for 7 days.

Jones et al. (1944) reported on the use of streptomycin to protect chicken embryos from the action of the fowl typhoid organism.

After completing two large experiments on the effect of treating turkeys suffering from fowl typhoid with furazolidone (NF-180), Boney (1954) reported this drug to be effective in reducing mortality due to fowl typhoid when fed at the rate of 2 pounds per ton of feed for 7 to 10 days, or until mortality stops. He also recommends the use of sanitary procedures after an outbreak.

Grumbles et al. (1954) found furazolidone added to the feed at levels of 0.0055 to 0.011 to be effective in preventing mortality associated with fowl typhoid (S. gallinarum) infection in turkeys. The higher level at 0.011 per cent was found to be more effective in severely infected birds. No evidence of toxicity or unpalatability was encountered. Exposed and treated birds have been shown susceptible to reinfection and to be carriers of S. gallinarum.

Richey (1954) reported that mortality was controlled promptly in field outbreaks of fowl typhoid in one chicken and two turkey flocks after feeding furazolidone (NF-180).

Glantz and Gordeuk (1955) report that in vitro and in vivo tests of sensitivity of Salmonella gallinarum to antibiotics were similar. Chloromycetin, when administered at the rate of 200 mg. per bird per day per os or 1 to 2 gm. per pound of mash, gave excellent protection when started on the day of infection. A relapse occurred when the chloromycetin mash was discontinued. Aureomycin mixed with mash at the rate of 1 gm. per pound reduced losses to 25 per cent and no relapse occurred when treatment was discontinued. Polymyxin B and Terracon 180 had little therapeutic value.

Smith (1955e) found furazolidone to be greatly superior to the sulfonamides in treatment of experimental fowl typhoid in one-day-old and nine-week-old chickens. This was thought to be due to the fact that furazolidone is bactericidal while the sulfonamides are bacteriostatic in low concentration. Penicillin was of no value in treatment.

Fecal excretion of Salmonella gallinarum could be stopped by feeding furazolidone in the mash at a concentration of 0.04 per cent for 10 days.

Chickens treated with furazolidone early but not late in the course of the experimental disease developed little immunity.

Furazolidone was of value in treatment of chronic carriers of infection.

Furazolidone, when fed continuously to young chicks in a low concentration (0.02 to 0.04 per cent), slightly depressed the growth rate but was not toxic.

Wilson (1955) found that furazolidone in a concentration of 0.02 per cent in the mash controlled mortality from Salmonella gallinarum infection but that mortality recurred after treatment ceased. At the 0.04 per cent level complete protection was obtained. When treatment was delayed until deaths occurred, results were less satisfactory but mortality was greatly reduced. Treatment begun at the earliest possible moment with 0.04 per cent of furazolidone and continued for 7 to 10

days, together with the practice of sanitary measures, is recommended.

Wilson (1956) concludes that the administration of furazolidone in the mash in the treatment of carriers will result in a proportion of birds being sterilized of Salmonella and ultimately becoming negative to the agglutination test. A larger percentage continue to harbor the organism, especially in distorted ova, and remain reactors. Treated birds usually cease laying Salmonella-containing eggs. Wilson further predicts that furazolidone may prove even more valuable in the prevention of salmonellosis in turkeys and ducks as direct egg transmission is common in these species.

Titkemeyer and Schmittle (1957) used various levels of six different drugs to determine their effects on the recovery of S. gallinarum from inoculated White Leghorn chicks. Representing the sulfa drugs, sulfaquinoxaline at levels of 0.05 per cent and 0.0175 per cent in the feed or at 0.025 per cent in the water and sulfamethazine at the level of 0.1 per cent in the water resulted in an incidence of recovery of 82.5 per cent as compared to 100 per cent in the inoculated controls. At "growth-promoting" levels, chlortetracycline at levels of 18 gm. and 108 gm. per ton and penicillin at 4 gm. per ton of feed did not result in a significantly lower rate of recovery of the organism. At therapeutic levels, chlortetracycline at 200 gm. per ton of feed resulted in 80 per cent recovery of the organism. With chlortetracycline in water, with neomycin alone or in combination with chlortetracycline in feed, the organism was recovered from 90 per cent of the chicks as compared to 100 per cent of the infected control. Furazolidone at the rate of 100 gm. per ton of feed reduced the incidence of recovery of the organism to only 10 per cent. With oral inoculation of the organism, the results were less conclusive in that often the organism could not be recovered regardless of whether the chick was medicated. Furazolidone seemingly prevented recovery of the organism in all 30 orally inoculated chicks. Time of administration of the medicaments whether 48 hours before, at time of, or 48 hours after, did not alter significantly the recoverability picture.

Dijkstra (1959) states that treatment of fowl typhoid with chemotheraputics and antibiotics nearly always gives unsatisfactory results because clinically healthy animals may remain carriers for a long time. Furazolidone, which has a bactericidal effect and apparently distinguishes itself favorably from the other drugs, cannot as a rule cure an infected flock.

Richey and Morgan (1959) artificially infected turkeys, 10 days old, with approximately 10^6 Salmonella gallinarum cells. While mortality in the untreated control group was 100 per cent, death losses were prevented by feeding chloramphenicol at 0.22 per cent, or furazolidone at 0.011 per cent or 0.022 per cent in feed starting 5 days before inoculation and continuing for 17 days.

When treatment was begun at the time of infection and continued for 12 days, deaths occurred in all groups. When medication was started four days after inoculation and continued for eight days, more birds treated with furazolidone than chloramphenicol survived. Chloramphenicol given at the rate of one 50-mg. capsule daily for three days was of little value in preventing deaths when given either at the time of inoculation or at the appearance of illness four days later.

Agglutination tests were negative in only one of the survivors medicated with chloramphenicol, but were negative in many of the survivors medicated with furazolidone.

Bierer et al. (1961) in four experiments found that prophylactic furaltadone water medication at 0.25 g/gal resulted in a marked reduction in mortality in pullorum disease in chicks, fowl typhoid in chicks and poults, and S. typhi-murium infection in poults.

Therapeutic medication at 0.25 g/ga

resulted in a reduction in mortality in all four trials. However, mortality reduction was marked on the 0.5 g/gal level in 3 of 4 trials but erratic results were obtained in poults with *S. typhi-murium* therapeutic medication.

In general, average body weights of all medicated groups at 10 days compared favorably with uninfected unmedicated controls, but were lower in the infected unmedicated groups.

Further evaluation of furaltadone water medication in more extensive floor pen trials, under field conditions, is indicated.

Hall and Cartrite (1961) investigated the resistance of some strains of *Salmonella gallinarum* to furazolidone and concluded that fowl typhoid is still a problem of major importance in Texas turkeys and chickens. Three of the four Texas Poultry Disease Investigation Laboratories reported one or more outbreaks of fowl typhoid that failed to respond to furazolidone therapy. Four isolates of *S. gallinarum* from such outbreaks were studied in the laboratory. All were sensitive *in vitro* to furazolidone; however, one strain developed resistance when serial passages were made in the presence of the drug. The same strain displayed resistance to the activity of furazolidone in experimentally infected turkeys when the drug was supplied in the feed at a level of 0.011 per cent (2 lb. NF 180 per ton).

Richey (1962) inoculated day-old chicks with *Salmonella pullorum* or *Salmonella gallinarum*. Medication with either of two soluble nitrofurans (nitrofurazone and furaltadone) in drinking water, alone and supplemented with furazolidone-medicated feed, prevented and reduced early (ten day) and total (four week) mortality in all but one group (infected with *S. pullorum* and treated with furaltadone).

Salmonella gallinarum-infected chicks succumbed rapidly. However, medication with furaltadone decreased both the early and total mortalities considerably. Appropriate time of medication rather than drug used accounted for the lowest number of infected survivors yielding a positive agglutination test.

In most cases, average weight of surviving *S. pullorum*-infected birds was higher than that of the birds infected with *S. gallinarum*.

Eradication

1. Elimination of carriers. Moore (1917), testing two flocks of typhoid-infected birds, one artificially and one naturally infected, found that they react intermittently just as they may with pullorum disease. He concluded, therefore, that fowl typhoid carriers may remain unnoticed if only one blood test is relied upon to remove all infected birds in a flock. He found that repeated testing at frequent intervals is necessary if all infected birds are to be detected, and that fowl typhoid reactors may appear perfectly healthy and continue to react month after month. Moore made a polyvalent *S. gallinarum* tube antigen which he considered superior to standard pullorum tube antigen in detecting carriers.

Holtman and Fisher (1917), in discussing the control of fowl typhoid, assert that the practice of a regular program of blood testing is an exceedingly important means of controlling the disease.

Hall *et al.* (1919b) were able to control extensive outbreaks of fowl typhoid in a large broiler and breeding flock establishment by repeated whole blood plate-agglutination tests and removal of reactors until no more were found. At first a gallinarum plate antigen was used, but later they concluded that the standard pullorum stained antigen was slightly superior to the gallinarum antigen in detecting carriers. When fowl typhoid first broke out in this establishment, control of the disease was attempted in house after house by depopulation of the affected and adjacent pens, followed by thorough cleaning and disinfection, but without success. It was possible to carry out only limited sanitary precautions at this plant; nevertheless, eradication of the disease was accomplished by the persistent elimination of car-

the Dry Antigen. The dry antigen is next combined with a slight amount of phenol salt solution (phenol 0.5 per cent, salt 0.85 per cent) and allowed to stand for two hours or longer to soften. Then the large, coarse, suspended particles are changed into a fine homogenous mixture by vigorous agitation for ten minutes with glass beads in a shaking apparatus.

Density of the bacterial test-suspension is determined by comparison with the usual standard density of barium sulfate (3 cc. of 1 per cent barium chloride solution and 97 cc. of 1 per cent sulphuric acid).

The *Salmonella gallinarum* dry antigen has been in use in the Giessener Institute since 1918. Up to the present time over a half million blood tests have been conducted in the vicinity of the poultry health service by the slow (tube) agglutination method. The antigen has proved satisfactory in all cases with regard to reaction capability, uniformity of composition and simplicity of preparation of the test-suspension. Also all comparative tests with the freshly prepared antigen (Taubitz, 1947; Zurich, 1919; and Geisler, 1958) recognize the clear superiority of the dry antigen.

Geissler (1958) states that agglutination antigens, which had been washed out by centrifugation and sedimentation, have a better reaction than "non-washed" bacteria. Dry antigen, following a method indicated by Lerche and Roots (1958) proves the same specificity and sensibility as cleaned, freshly prepared antigens. The dry antigen, which can always be produced in the same quality, seems to be the best reagent.

Molnar and Nagy (1959) describe the production of fowl typhoid antigen by fermentation, viz.: *S. gallinarum* may be readily cultivated by fermentation using a liquid medium containing hydrolyzed casein. As the result of investigations the propagation of bacteria was finished after 7-8 hours' culture and by this time the culture contained 120 to 150 milliards of germs per ml. These cultures proved suitable for the preparation of fowl typhoid antigen. As a result of comparative serological tests, using blood samples from both naturally and artificially infected birds, the value of the antigen prepared by fermentation was similar to that prepared from suspensions of agar cultures.

2. Depopulation. Depopulation is the traditional method of eradicating disease, and may be used in small flocks kept for egg or meat production. When followed by a thorough cleaning and disinfection, or by a sufficient interval of vacancy, such a procedure is successful, provided healthy replacement stock is obtained. This method of disease eradication, however, is too costly in most breeding flocks where much expense and years of effort have been devoted to the development of birds of superior quality. Control by depopulation is an emergency measure and indicates that the disease has been insufficiently studied to provide a satisfactory control method.

REFERENCES

Alberts, J. O.: 1950. The prophylactic and therapeutic properties of sulfamerazine in fowl typhoid. Am. Jour. Vet. Res. 11:421.

Beach, J. R., and Davis, D. E.: 1927 Acute infection of chicks and chronic infection of the ovaries of hens caused by the fowl typhoid organism. Hilgardia 2:411.

Beaudette, F. R.: 1925. The possible transmission of fowl typhoid through the egg. Jour. Am. Vet. Med. Assn 67:741.

———: 1930. Fowl typhoid and bacillary white diarrhea. Proc. 11th Internat. Vet. Cong. London. Part 3:705.

———: 1938. An outbreak of fowl typhoid in guineas. Jour. Am. Vet. Med. Assn. 92.695.

Beck, A., and Eber, R.: 1929. Die wichtigsten bakteriellen Kückenerkrankungen, ihre Diagnose, Differentialdiagnose und Bekämpfung. Zeitschr. f. Infekti-Krankh. d. Haustiere 35:76.

Bierer, B. W., Valentine, H. D., and Vickers, C. L.: 1961 Furaltadone water medication: Its use in avian Salmonellosis. Avian Dis. 5 214.

Bigland, C. H.: 1954. Fowl typhoid control in Alberta. Proc. A.V.M.A. 91st Ann. Meet., Aug. 23–26, P. 340.

Blaxland, J. D., Sojka, W. J., and Smither, A. M.: 1956. A study of S. pullorum and S. gallinarum strains isolated from field outbreaks of disease. Jour. Comp. Pathology and Therap. 66:270.

Boney, W. A., Jr.: 1947. Isolation of Shigella gallinarum from turkey eggs. Am. Jour. Vet. Res 8:133.

———: 1954. Fowl typhoid yields to new drug. Turkey World. (Feb.) P. 40.

Botts, C. W., Ferguson, L. C., Birkeland, J. M., and Winter, A. R.: 1952. The influence of litter on the control of Salmonella infections in chicks. Am. Jour. Vet. Res. 13:562.

Brown, H. C., Duncan, J. T., and Henry, T. A.: 1924. The fermentation of salts of organic acids as an aid to the differentiation of bacterial types. Jour. Hyg. (London) 23:1.

Bushnell, L. D., and Patton, J. W.: 1924. The use of vaccines in poultry diseases. Poultry Sci. 4.61.

Chubb, L. G., Gordon, R. F., and Tucker, J. F.: 1958. The effect of high levels of vitamins on the susceptibility of chickens to Salmonella gallinarum infection. Brit. Vet. Jour. 114.55.

Cloud, C. F.: 1943. Perforation with peritonitis from Shigella gallinarum (var. Duisburg). Med Bul. Veterans' Administration. 19:335.

Coles, J.D.W.A.: 1946. Fowl typhoid. South African Poultry, Pigeon, and Bird Magazine 52:199

Cruickshank, G. A.: 1927. Employment of a double sugar medium for routine diagnosis of bacillary white diarrhea, fowl typhoid, and fowl cholera. Jour. Bact. 14:435.

Curtice, G.: 1902. Fowl Typhoid. R. I. Agr. Expr. Sta. Bul. 87.

Dearstyne, R. S., Greaves, R. E., and Gauger, H. C.: 1933. The control of Avian typhoid by the use of bacterins. Proc. Fifth World's Poultry Cong. (Rome). Part 3:108.

Delpy, L., and Rastegar, Kt. 1935. Étude de souches, américains, asiatiques et européennes de microbes du groupe pullorum-gallinarum Ann. de l'Inst. Past. 61:586.

d'Herelle, F.: 1919. Sur le rôle du microbe bactériophage dans la typhose aviaire. Compt. rend. Acad. Sci. 169:932.

———: 1922. The Bacteriophage; Its Role in Immunity. Pp. 206–7. The Williams and Wilkins Co., Baltimore.

Dijkstra, R. G.: 1959. Fowl typhoid: a review. Tijdschr. Diergeneesk. 84:575.

Donatien, A., Plantureaux, E., and Lestoquard, F.: 1923. La typhose aviare en Algérie. Ann. de l'Inst. Past. d'Algérie I:585.

Edwards, P. R.: 1928. The fermentation of maltose by Bacterium pullorum. Jour. Bact. 15 235.

———: 1939. Standard strains of Salmonella. Ky. Agr. Exper. Sta., Circ. 50.

El-Dine, H. S.: 1959. Important diseases of poultry in Egypt and their control. Proc. Seventh World's Poultry Cong., P. 229.

Eurich, G.: 1949. Beitrag zur Bekämpfung der bazillaren Kukenruhr (Pullorum-Infektion) mittels serologischer Untersuchung der Zucht-huhner. Inaug.-Disert., Giessen.

Fox, H.: 1923. Diseases of Captive Wild Animals and Birds. First Edition. Lippincott Co., Philadelphia. P. 598.

Garsen, H. W., and Hill, C. H.: 1959. Agglutinating antibody titers of young White Leghorns and Rhode Island Reds following inoculation with live and inactivated Salmonella gallinarum cultures. Poult. Sci. 38:918.

Gauger, H. C.: 1934. A chronic carrier of fowl typhoid with testicular focalization. Jour. Am. Vet. Med. Assn. 84:218.

Geissler, Von H.: 1958. Vergleiche der agglutinations ergebnisse verschieden hergestellter antigene bei der Infektion mit Sal. gallinarum-pullorum. Berl. und Munch. tierarztl. Wochenschr. 71-828.

Glantz, P. J., and Gordeuk, S., Jr.: 1955. In vitro and in vivo sensitivity of the fowl typhoid organism, S. gallinarum to antibiotics. Poultry Sci. 34:880.

Glover, J. A., and Henderson, W.: 1946. Fowl typhoid. Report on a recent outbreak in Ontario. Jour. Comp. Med. 10-241.

Goldberg, S. A.: 1917. A study of the fermenting properties of B. pullorum (Rettger) and B. sanguinarium (Moore). Jour. Am. Vet. Med. Assn. 51:203.

Gordeuk, S., Jr., Glantz, P. J., Cattenbach, E. W., and Thorp, W. T. S.: 1949. Transmission of fowl typhoid. Poultry Sci. 28:885.

Gordon, R. F., Garside, J. S., and Tucker, J. F.: 1959. The use of living attenuated vaccines in the control of fowl typhoid. Vet. Rec. 71:300.

Gordon, W. A. M., and Luke, D.: 1958. A note on the use of 9R fowl typhoid vaccine in poultry breeding flocks. Vet. Rec. 71:926.

Goret, P., Joubert, L., and Oudar, J.: 1956. Invalidity of the distinction between Salmonella gallinarum and Salmonella pullorum. Biochemical variants of these organisms. Ann. Inst. Past. 90 A. [Abstracts—A.V.M.A. Jour. 130:423, May 1, 1957. J. P. Scott.]

Grumbles, L. G., Wills, F. K., and Boney, W. A., Jr.: 1954. Furazolidone in the treatment of fowl typhoid in turkeys. Jour. Am. Vet. Med. Assn. 124:217.

Gwatkin, R., and Dzenis, L.: 1951a. Fowl typhoid. I. Comparison of antigenicity of sixteen gallinarum antigens. Canad. Jour. Comp. Med. and Vet. Sci. 15.15.

Hadley, P., Caldwell, D. W., Elkins, M. W., and Lambert, D. J.: 1917. Infections caused by Bacterium pullorum in adult fowls. R. I. Agr. Exper. Sta., Bul. 172.

Hall, C. J., and Cartrite, H. T.: 1961. Observations on strains of *Salmonella gallinarum* apparently resistant to furazolidone. Avian Dis. 5:382.

Hall, W. J.: 1946. Fowl Typhoid. Circ. No. 755. U.S.D.A. Pp. 1–9.

———. Legenhausen, D. H., and MacDonald, A. D.: 1918. A summary of results of fowl typhoid investigations. Proc. 20th Annual Meeting of the Northeast Conference of Laboratory Workers in Pullorum Dis. Control.

———. Legenhausen, D. H., and MacDonald, A. D.: 1919a. Studies on fowl typhoid. 1. Nature and dissemination. Poultry Sci. 28:344.

———. MacDonald, A. D., and Legenhausen, D. H.: 1919b. Studies on fowl typhoid. 11. Control of the disease. Poultry Sci. 28.789.

Hammond, J. C.: 1946. Sulfonamides in the control of fowl typhoid. Poultry Sci. 24:382.

Harbourne, J. F.: 1957. The control of fowl typhoid in the field by the use of live vaccines. Vet. Rec. 69.1102.

Haupt, H.: 1935. IV. Zur Systematik der Bakterien. Die für Mensch und Tier pathogenen gramnegativen alkalibildenden Stäbchenbakterien. Ergeb. Hyg. Bakt. Immunit. u. Exper. Therap. 17:175.

Hendrickson, J. M.: 1927. The differentiation of *Bacterium pullorum* (Rettger) and *Bacterium sanguinarium* (Moore). Jour. Am. Vet. Med. Assn. 70.629.

Hill, C. H., and Garren, H. W.: 1955. The effect of high levels of vitamins on the resistance of chicks to fowl typhoid. Ann. N.Y. Acad. Sci. 63:186.

———. and Garren, H. W.: 1958. Plasma ascorbic acid levels of chicks with fowl typhoid. Poultry Sci. 37:236.

———. and Garren, H. W.: 1961. Protein levels and survival time of chicks infected with *Salmonella gallinarum*. Jour. Nutr. 73:28.

Hinshaw, W. R.: 1930. Fowl typhoid of turkeys. Vet. Med. 25:514.

———. 1941. Cysteine and related compounds for differentiating members of the genus Salmonella. Hilgardia 13.583.

———. and Rettger, L. F.: 1936. Cysteine-gelatin as a differential medium for *Salmonella pullorum* and *Salmonella gallinarum*. Proc. Soc. Exper. Biol. and Med. 35:44.

———. and Taylor, T. J.: 1933. A chronic carrier of fowl typhoid of turkeys. Jour. Am. Vet. Med. Assn. 82:292.

Holtman, D. F., and Fisher, G.: 1946. Some observations on the control of fowl typhoid infection with sulfa drugs. Jour. Bact. 51:401.

———. and Fisher, G.: 1947. The application of sulfonamides to the control of typhoid in poultry. Poultry Sci. 26:478.

Hudson, C. B., and Beaudette, F. R.: 1929. The isolation of *Bacterium pullorum* from a European bullfinch (*Pyrrhula europa*). Jour. Am. Vet. Med. Assn. 74:529.

Ishii, T., Sakazaki, R., and Watanabe, Y.: 1938. Distinction of *Salmonella gallinarum* and *S. pullorum*. Bul. Nat. Inst. Anim. Health, Tokyo, 35:21.

Johnson, E. A., and Rettger, L. F.: 1912. A comparative study of the nutritional requirements of *Salmonella pullorum*, *Salmonella gallinarum*, and *Salmonella typhosa*. Jour. Bact. 43:103.

Johnson, E. P., and Anderson, G. W.: 1933. An outbreak of fowl typhoid in guinea fowls. (*Numida meleagris*). Jour. Am. Vet. Med. Assn. 82:258.

———. and Pollard, M.: 1940. Fowl typhoid in turkey poults. Jour. Am. Vet. Med. Assn. 96:243.

Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A.: 1944. Control of Gram-negative bacteria in experimental animals by streptomycin. Science 100:103.

Jordan, E. O., and Harmon, P. H.: 1928. A new differential medium for the paratyphoid group. Jour. Infect. Dis. 42:238.

Jordan, F. T. W.: 1956a. The occurrence of *S. gallinarum* in the feces in fowl typhoid. Poultry Sci. 35:1026.

———. 1956b. The transmission of *S. gallinarum* through the egg. Poultry Sci. 35:1019.

Kauffmann, F.: 1930. Die Technik der Typenbestimmung in der Typhus-Paratyphus Gruppe. Zentralbl. f. Bakt. I. Orig. 119:152.

———. 1934. Untersuchungen über die Duisburger Gallinarum Stämme. *Ibid.* 132:337.

Kaupp, B. F., and Dearstyne, R. S.: 1924a. Chronic carriers of fowl typhoid. Jour. Am. Vet. Med. Assn. 64:329.

———. and Dearstyne, R. S.: 1924b. Fowl typhoid. A comparison of various European strains with those of North America. Poultry Sci. 3:119.

———. and Dearstyne, R. S.: 1925. The differential diagnosis of fowl cholera and fowl typhoid. Jour. Am. Vet. Med. Assn. 67:249.

Kerr, W. R.: 1930. Selective media for the cultivation of *Bacillus pullorum* and *B. sanguinarium*. Jour. Comp. Pathology and Therap. 43:77.

Klein, E.: 1889. Ueber eine epidemische Krankheit der Hühner, verursacht durch einen Bacillus—*Bacillus gallinarum*. Zentralbl. f. Bakt. 5.689.

Klimmer, M., and Haupt, H.: 1927. Ueber Infektion von Hühnern mit dem *Bacterium gallinarum* Klein (1889). Zentralbl. f. Bakt. I. Orig. 105:99.

Komarov, A.: 1932. Der Kennzeichen eines hohe chicks. Vet. Record 12:1455.

Kraus, E. J.: 1918. Zur Kenntnis des Hühnertyphus. Zentralbl. f. Bakt. I. Orig. 82.282.

Lambert, W. V.: 1933. A preliminary study of the reaction in two disease resistant stocks of chickens after infection with their reciprocal pathogens. Iowa Acad. Sci. 40:231.

Lerche, Von M.: 1939. Salmonellainfektionen beim Geflugel und ihre Bedeutung für die Epidemiologie der Salmonellabakterien. Proc. Seventh World's Poultry Cong., p. 274.

———, and Roots, E.: 1958. Herstellung des Salmonella gallinarum—Trockenantigens für die Agglutinationsreaktion. Berl. und Munch. tierarztl. Wochenschr. 71:431.

Lignières, J., and Zabala: 1903. Sur une nouvelle maladie des poules. Bul. Soc. Cent. de Méd. Vét. 59:453.

Lucet, A.: 1891. Dysenterie épizootique des poules et des dindes. Ann. de l'Inst. Past. 5:312.

Machan, C.: 1959. Control of Salmonella pullorum and S. gallinarum infection in Austria. Wien. tierarztl. Monatschr. 46:190.

McNutt, S. H.: 1926. Vaccination of poultry. Jour. Am. Vet. Med. Assn. 69:472.

Mallmann, W. L.: 1931a. Studies on bacteriophage in relation to salmonella and pullorum disease. Mich. Agr. Exper. Sta., Bul. 109.

——: 1931b. Use of organic acids for the differentiation of Salmonella pullorum and Salmonella gallinarum. Proc. Soc. Exper. Biol. and Med. 28:501.

———, and Snyder, D.: 1929. Differential medium for Salmonella pullorum, Salmonella gallinarum, Pasteurella avicida, and Escherichia coli Jour. Infect. Dis 44:13.

———, Thorp, F.A. and Semmer, M.: 1928. A medium for the isolation of Salmonella pullorum and other members of the paratyphoid group from avian tissues. Jour. Am. Vet. Med. Assn. 73:825.

Manninger, R.: 1930. Huhner typhus und bakterielle Kückenruhr Proc. 11th Internat. Vet. Cong. (London), Part 3:724.

Martinaglia, G.: 1929. A note on Salmonella gallinarum infection of ten-day old chicks and adult turkeys. Jour. So. Africa Vet. Med. Assn. 1:35.

Miessner, H.: 1930. Die Pullorum Infektion der Huhner (Die weisse Ruhr des Kücken-Hühnertyphus). Deutsch. tierärztl. Wochenschr. 38:517.

Molnar, I., and Nagy, G.: 1959. Production of fowl typhoid antigen by fermentation. Mag afbator. Lapja 14:386.

Monteverde, J. J., and Simeone, D. N.: 1944. Salmonelas genuinamente aviárias en aves "reaction antes." Univ. Buenos Aires Fac. Agron. y Vet. Inst. etc. 1–50 (Biol. Abs, Vol. 19, 1945).

Moore, E. N.: 1946a. Fowl typhoid transmission. Del. Agr. Exper. Sta., Bul. 262.

——: 1946b. The occurrence of fowl typhoid. Del. Agr. Exper. Sta., Circ. 19.

——: 1946c. The efficacy of recently developed sulfonamides against fowl typhoid. Poultry Sci 25:507.

——: 1947. The agglutination test as a means of detecting fowl typhoid infection. Cornell Vet. 37:21.

Moore, V. A.: 1895. Infectious leukemia in fowls—A bacterial disease frequently mistaken for fowl cholera. U.S.D.A. Bur. Anim. Ind., 12th and 15th Ann. Rep.

Mulsow, F. W.: 1919. The differentiation and distribution of the paratyphoid-enteritidis group. IV. Avian paratyphoid bacilli: A comparative study of B. pullorum and B. sanguinarium. Jour. Infect. Dis. 25:135.

Munné, J. V.: 1937. Au sujet de la différentiation de Salmonella pullorum et S. sanguinarium au moyen d'un bactériophage spécifique. Compt. Rend. Soc. de Biol. 126:1226.

Nóbrega, P.: 1935. Differenciacao entre "S. pullorum" e "S. gallinarum." Papel importante do bacteriophago. Arq. do Inst. Biol.—São Paulo 6:71.

——, and Bueno, R. C.: 1942. Sobre a Presenca da Salmonella gallinarum nos ovos de galinhas portadoras de tifo aviairo. Arq. do Inst. Biol.—São Paulo 13:17.

Orr, Betty B., and Moore, E. N.: 1955. Longevity of S. gallinarum. Poultry Sci. 32:800.

Pacheco, C., and Rodrigues, C.: 1935a. Nouveau representant des bacteries du groupe pullorum-gallinarum. Morphologie des colonies du groupe. Compt. Rend. Soc. de Biol. 118:905.

———, and Rodrigues, C.: 1935b. Biologie des bacteries du groupe pullorum-gallinarum. Action sur les milieux au lait et sur le rouge neutre. Ibid. 118:1019.

———, and Rodrigues, C.: 1936. Biologie du groupe pullorum-gallinarum. Caractérisation des types qui composent le groupe. Ibid. 121:590.

Palmer, C. C., and Baker, H. R.: 1928. Fowl typhoid. Del. Agr. Exper. Sta., Bul. 153.

Panisset, L.: 1930. Bacillary white diarrhea and fowl typhoid. Proc. 11th Internat. Vet. Cong., (London). Part 3:741.

Pfeiler, W.: 1920. Identitatsnachweis für die Erreger der Kleinischen Huhnerseuche und des Pfeiler-Rehsesschen-Hühner typhus Bazillus. Zentralbl. f. Bakt. I. Orig. 85:193.

———, and Rehse, A.: 1913. Bacillus typhi gallinarum alcalifaciens und die durch ihn verursachte Huhnerseuche. Mitt. a.d. Kaiser Wilhelm Institut f. Landwirtschaft zu Bromberg. 5:506.

———, and Roepke, W.: 1917. Zweite Mitteilung uber das Auftreten des Hühnertyphus und die Eigenschaften seines Erregers. Zentralbl. f. Bakt. I. Orig. 79:125.

Rao, S. B. V., Narayanan, S. Ramnani, D. R., and Das, J.: 1952. Avian salmonellosis: Studies on Salmonella gallinarum. Indian Jour. Vet. Sci. and Anim. Husb. 22:199.

Rettger, L. F., and Koser, S. A.: 1917 A comparative study of Bacterium pullorum (Rettger) and Bacterium sanguinarium (Moore). Jour. Med. Res. 35:443.

Richey, D. J.: 1954. Furazolidone shows promise for control of fowl typhoid and pullorum disease. S.C. Agr. Res. 1:1.

Richey, D J.: 1962. Water soluble nitrofuran therapy in pullorum and fowl typhoid in chicks. Am. Jour. Vet. Res. 23:102.

―――, and Morgan, C. L.: 1954. Treatment of Salmonella gallinarum infection in turkey poults with chloramphenicol and furazolidone. Am. Jour. Vet. Res. 20.659.

Rodrigues, C., and Pacheco, G.: 1936 Biologie du groupe pullorum gallinarum. Observations relative à la différentiation des types pullorum par la fermentation de la maltose. Compt. Rend Soc de Biol 123 458

St. Johns Brooks, R., and Rhodes, M: 1923. The organism of the fowl typhoid group. Jour. Pathology and Bact 26-433

Simms, B T. 1946. Tests of drugs and vaccine to control fowl typhoid. Rep. Chief Bur. Anim. Ind. Agr Res Adm. U.S.D.A., p. 40.

Smith, W. V.: 1954. Food as a vehicle of infection: The effect of variations in the diet on the induction of Salmonella gallinarum infection. Brit. Jour. Exper. Pathology 35:447.

―――― 1955a The longevity of S gallinarum in the feces of infected chickens. Jour. Comp. Pathology and Therap. 65.267

―――― 1955b Observations on experimental fowl typhoid. Jour. Comp. Pathology and Therap. 65:37.

―――― 1955c The chemotherapy of experimental fowl typhoid in fowls (Gallus domesticus). Jour. Comp Pathology and Therap 65 55.

―――― 1956. The use of live vaccines in experimental S. gallinarum infection in chickens with observations on their interference effect. Jour. Hyg. 54:419.

――――, and Chubb, I. C. 1957 The effect of feeding different levels of protein concentrates on the susceptibility of chickens to S gallinarum infection. Jour. Comp. Pathology and Therap. 67-10.

Smith, Th. 1915 A note on the relation between B. pullorum (Rettger) and the fowl typhoid bacillus (Moore) Jour Med Res 31 547.

――――, and Ten Broeck, C: 1915. Agglutination affinities of a pathogenic bacillus from fowls (fowl typhoid) (Bact. sanguinarium, Moore) with the typhoid bacillus of man. Jour. Med. Res 31:503.

Snoeyenbos, G H., Bullis, K. L., and Van Roekel, H.: 1955. Some essentials for the control of fowl typhoid Proc 27th Ann. Meeting of Northeastern Conf. of Laboratory Workers in Pullorum Dis Control June 14 15

Taubitz, K. 1947. Vergleichende Blutuntersuchungen beim Huhn auf Bact. Pullorum unter Verwendung verschiedener Teste. Inaug. Dissert., Berlin.

te Hennepe, B. J C. 1924 Combating poultry diseases by the state serum institute at Rotterdam. Proc Second World's Poultry Cong., p 219.

―――― 1939 Combating poultry diseases in the Netherlands. Proc. Seventh World's Poultry Cong., p. 224.

――――, and van Straaten, H: 1921. Fowl septicemia Trans. First World's Poultry Cong. 1:289.

Tittsemeyer, C W, and Schmutte, S C: 1957. The effect of drugs on the isolation of Salmonella gallinarum from inoculated chicks Poultry Sci. 36:1193.

Trabulsi, L. R. and Edwards, P R · 1962. The differentiation of Salmonella pullorum and Salmonella gallinarum by biochemical methods. Cornell Vet. 52:563.

Truche, C: 1925 De la typhose aviaire. Ann de l'Inst. Past. 37:478.

Van Es, L., and Olney, J. F. 1940. An inquiry into the influence of environment on the incidence of poultry diseases Fowl typhus Univ of Neb., Res. Bul. 118.

van Heelsbergen, T. 1929 Handbuch der Geflugelkrankheiten und Geflugelzucht. Ferdinand Enke, Stuttgart P 135

Van Roekel, H: 1955. A study of the variation of Salmonella pullorum. Mass. Agr. Exper. Sta. Bul. 319

―――― 1937 Maltose fermenting S pullorum strains. Mass. Agr. Exper. Sta., Bul. 339.

―――― 1962 Additional measures required for the eradication of pullorum-typhoid infections. Avian Dis. 6.178.

van Straaten, H., and te Hennepe, B. J C 1918. Die Kleinsche Huhnerseuche. Folia Microbiology 5-103.

Wagener, K.: 1954. Kuckenruhr. Proc. 12th Internat. Vet. Cong. (New York). Part 3.108.

Ward, A. R., and Gallagher, B A 1920. Diseases of Domesticated Birds. Macmillan Co., New York.

Williams, J. E., and Harris, M. E.: 1956 Antigenic studies using ammonium sulfate. IV. The sedimentation effect of ammonium sulfate on Salmonella gallinarum. Am. Jour. Vet. Res. 17:535.

Wilson, J. E. 1946 Fowl typhoid: Certain aspects of the experimentally produced disease. Vet. Record 58:269

―――― 1955. The use of furazolidone in the treatment of infections of day-old chicks with S. pullorum, S. gallinarum, S. typhimurium, and S. thompson. Vet. Record 67.849.

―――― 1956. The treatment of carriers of S pullorum and S gallinarum with furazolidone. Vet. Record 68:748.

―――― 1958. Fowl typhoid in Great Britain. Agr. Rev., London 3-23.

Winmill, A. J.: 1961. Control of fowl typhoid in Kenya. Bul. Epiz. Dis. Afr 9.383.

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11

Fowl Cholera

Fowl cholera (avian pasteurellosis) is an infectious disease affecting practically all species of fowls. It usually appears as a septicemic disease associated with high morbidity and mortality, but chronic manifestations are also of frequent occurrence.

History. According to Gray (1913), various epizootics among fowls occurred in European countries during the latter half of the eighteenth century, and he credited Chabert with the first study of this disease in France in 1782. Maillet, in 1836, cited by Manninger (1929), was the first to use the designation "fowl cholera" in connection with severe losses. Renault and Delafond presented the first experimental evidence of the transmissibility of fowl cholera about the middle of the nineteenth century, according to Manninger.

Rivolta, in 1877, and Perroncito, in 1878, described the presence of microbes having a rounded form, appearing singly, or two combined, in the blood of affected birds.

Toussaint confirmed these observations in 1879 and demonstrated that the organisms were the cause of the disease; he obtained cultures in neutralized urine (Gray, 1913). Pasteur (1880a) isolated and grew pure cultures of the microorganism in chicken broth. In continuing studies, Pasteur (1880b), using fowl cholera organisms, performed his fundamental experiments in the attenuation of bacteria in culture and their use in inducing immunity.

Salmon (1880, 1881, 1883) appears to have been the first to have investigated fowl cholera in the United States, and Higgins (1898) had an early report of disease in Canada.

Incidence. Fowl cholera occurs sporadically or enzootically in most countries of the world, although it is of more frequent occurrence in temperate and warm regions than in northern countries. At times it causes a heavy mortality; at others the losses are nominal. There is evidence of a decrease in the incidence of fowl cholera

[359]

in several countries since about 1930. Van den Hurk (1950) reported that fowl cholera is uncommon in Holland. Woolbridge (1954) stated that the acute type of the disease is not normally present in Great Britain, and outbreaks are usually traced to importations. In Denmark, the incidence of fowl cholera declined sharply following bans on importation of live poultry (Marthedal and Velling, 1954). Iyer and Hashmi (1945) reported that the disease occurs in India far less frequently than is usually believed, and that widespread epizootics are not a feature of the disease in that country.

In the United States, many of the poultry diagnostic laboratories have noted a reduced incidence of fowl cholera in recent years, finding only a few sporadic outbreaks. The decreased incidence may well be associated with general improvements in poultry management practices. On the other hand, Eveleth et al. (1951) reported that the disease is widespread in North Dakota in both chicken and turkey flocks, occurring during the entire year but especially at times of management changes. They stated that the disease appeared to be on the increase. Dorsey and Harshfield (1959) ranked fowl cholera among the three most important diseases of poultry in South Dakota. The highest incidence occurred in the months from August through December. Bierer (1962) reported that pasteurellosis in chickens in South Carolina and adjoining areas exists mainly as a persistent subacute and chronic disease that clinically resembles avian monocytosis and that acute fowl cholera is rarely observed. Hagan and Bruner (1961) reported heavy losses of ducklings on the duck "ranches" of Long Island where the birds are raised in large numbers in very crowded insanitary quarters.

Etiology. *Pasteurella multocida* (*P. avicida*, *P. aviseptica*, *P. choleraegallinarum*, etc.), the causative agent of fowl cholera, is a small nonmotile, Gram-negative, ovoid or elongated rod (Fig. 11.1). It measures 0.25–0.4μ by 0.6–2.5μ but tends towards

pleomorphicity after repeated culture on agar, or prolonged cultivation in broth or carbohydrate media. A capsular substance of a mucoid nature may be demonstrated in recently isolated cultures. In tissues, blood, and recently isolated cultures on blood agar the organism is distinctly bipolar when carefully stained with methylene blue, Giemsa, or carbol-fuchsin. This characteristic is lost with continued cultivation on artificial media.

On beef infusion media the colonies of *P. multocida* are round, flat, and translucent. Fluorescence is noted in colonies of many recently isolated strains. In broth the organism produces a diffuse clouding. A sticky sediment collects in the bottom of old broth cultures. With some strains a flocculent precipitate may be noted which is characteristic of a rough phase of the organism. The addition of sterile serum and use of digested protein or proteose peptone stimulates growth in solid and liquid media.

P. multocida is an aerobe or facultative anaerobe with an optimum growth temperature of 37° C. The optimum pH for growth is 7.2 to 7.4 with a range of 6 to 8.5. It produces indol, catalase, and ammonia. It reduces nitrates to nitrites, reduces methylene blue, does not produce methyl acetyl carbonol, and is negative to
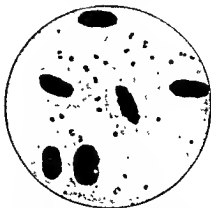
FIG. 11.1 – *Pasteurella multocida*, blood smear, fowl cholera. ×2,000. (From Nowak, *Documenta Microbiologica*, Gustav Fischer.)

the methyl red test. It is not bile-soluble, does not liquefy gelatin or change litmus milk (Merchant and Packer, 1961).

Rosenbusch and Merchant (1939) classified *P. multocida* strains into three groups on the basis of their fermentation of xylose, arabinose, and dulcitol. Group I fermented arabinose and dulcitol but not xylose; Group II fermented xylose but not arabinose and dulcitol. A third group was variable but more nearly like Group I. Dextrose, mannose, galactose, saccharose, and mannitol were fermented by both main groups, but not lactose, maltose, raffinose, trehalose, rhamnose, inositol, adonitol, inulin, salicin, dextrin, and starch. Dorsey (1963a) made a similar study of 409 *P. multocida* strains, all of fowl origin. Three hundred thirty-three (81.42 per cent) were Group I and 69 (16.87 per cent) were Group II. Seven strains (1.71 per cent) which fermented xylose, arabinose, and dulcitol were classified as Group III. Twenty-three additional strains varied from the reactions typical of the three groups. Usually there was correlation between the biochemical group and the agglutinative and immunizing actions of the strains but exceptions were encountered (Dorsey, 1963b).

Roberts (1947) arrived at another classification, recognizing 4 types of *P. multocida* based on immunological differences in cross-protection experiments with mice. Little and Lyon (1943) studied 30 strains of *P. multocida* serologically, dividing them into 3 types. Carter (1955) examined numerous strains by means of hemagglutination tests and proposed 4 types which were designated A, B, C, and D. The following table relates Carter's classification with the earlier designations:

Carter	Types	A	B	C	D
Rosenbusch and Merchant	Groups	I	II		
Little and Lyon	Types	1	2	3	
Roberts	Types	II	I	III	IV

Namioka and Murata (1961a) employed a slide agglutination test in comparison with hemagglutination in capsule typing and found the former satisfactory for cultures with fluorescent characteristics. In continuing studies (Namioka and Murata, 1961b, c; Namioka and Bruner, 1963) attention was given, also, to the somatic (O) antigen of *P. multocida*. Ten O groups were demonstrated within 156 cultures. By correlation of these O groups with the capsule types, 12 serotypes were delineated. Almost all of serotype 5:A cultures were recovered from fowl cholera. Both 5:A and 9:A serotypes were isolated from fowl cholera in turkeys. A few cultures from fowls were O group 2 or 4 and two were of unidentified O group. Two strains of *P. multocida* used by Heddleston (1962) to produce bivalent fowl cholera bacterin were identified as serotypes 5:A and 8:A. Serotypes 5:A, 8:A and 9:A proved pathogenic while other serotypes belonging to capsule groups A, B, and D had little pathogenicity for 3 month-old chickens.

Hughes (1930), studied the colony morphology of 210 freshly isolated strains of *P. multocida*, distinguishing 3 variations. One type, "fluorescent," was associated with outbreaks of acute fowl cholera and was highly virulent. The second type, "blue," occurring in flocks in which cholera was endemic, was of low virulence. A third type, "intermediate," was associated with more severe cholera and was intermediate in behavior.

Carter (1955) described three principal colonial variants of *P. multocida* as follows: (1) mucoid (M) with large flowing colonies on agar, a mucoid deposit in broth, and with moderate mouse virulence; (2) smooth (fluorescent) (F) with medium-sized discrete colonies, diffuse growth in broth, and with high mouse virulence; (3) rough (blue) (I) with small, discrete colonies, showing autoagglutination in broth, and having low virulence for mice. Many of the cultures recovered from carrier fowls and animals, and from chronic processes, were composed of predominantly M variants, and were not typable by agglutination, precipitation, or capsular

TABLE 11.1
Classification of Colonial Variants of *P. multocida*

| Older Designations | Proposed Designations | | Capsule | Reaction in Acriflavine |
	Colonial	Antigenic		
Mucoid........ ..	mucoid	M	+ +	slimy precipitate
Fluorescent... ..	smooth	S	+	cells remain in suspension
Intermediate.... ..	smooth	S	+	partial flocculation
Granular Blue	smooth	SR		flocculation
Rough.... .	rough	R		flocculation

swelling techniques. In a later report, Carter (1957) gave further attention to colonial variation and antigenic characteristics of *P. multocida*. Colony morphology was studied by obliquely transmitted light, and antigenic characteristics by behavior with 1:1000 acriflavine solution. His proposed designations were related to older designations as shown in Table 11.1.

Hall *et al.* (1955) described a microorganism isolated from chickens with chronic fowl cholera and chickens with upper respiratory infection, which differed from other members of the Pasteurella group. It produced acid in maltose, trehalose, and mannose, was nonhemolytic, and did not produce indol. The name *Pasteurella gallinarum* was proposed for this new species. Gibbs (1910) had earlier isolated from ducks an organism resembling *P. multocida* which was maltose-positive and indol-negative. Hemolytic Pasteurellae have been isolated from various types of lesions in chickens and turkeys by Greenham and Hill (1962), Harbourne (1962), and Harry (1962).

Tenacity. *Pasteurella multocida* is readily destroyed by 3 per cent cresol, 1 per cent phenol, and 1:5000 bichloride of mercury. At 60° C., the organism is destroyed in 10 minutes. It remains viable in manure for at least a month (Gartner, 1898) and about 3 months in the decaying carcass and in garden soil (Kitt, 1888). The organism survived in dried blood smears on glass for 8 days at room temperature (Skidmore, 1932).

Van Es and Olney (1940), in studies of the influence of environment on the incidence of fowl cholera, found that the infection hazard had apparently disappeared from a poultry yard two weeks after the occurrence of the last death and the removal of birds.

The influence of temperature on the viability and virulence of *P. multocida* in cultures has been studied by Nobrega and Bueno (1950). Broth cultures stored in sealed tubes at an average room temperature of 17.6° C. were still viable and virulent after two years. At temperatures of 2 to 4° C. such cultures were inactive at the end of one year. Sealed agar slant cultures were held at room temperature for 3 years or longer with only a few strains becoming nonviable (Dorsey and Harshfield, 1959).

Susceptibility. Domestic fowls of all species, game birds raised in captivity, and small feral birds (pigeons, sparrows, finches, etc.) that may visit poultry yards are susceptible to fowl cholera. Most of the reported outbreaks of fowl cholera have involved chickens, turkeys, and ducks, and studies of the disease have more often concerned those species. Curtice (1902) described a heavy loss in 1900 in Rhode Island due to "goose septicemia." About 3,200 geese died out of a flock of 4,000 in a short period of time. Others (Moore, 1916; Ward and Gallagher, 1920) used

the name "goose septicemia" for Pasteurella infections in geese, although the separate identity was apparently based on its peracute nature and the very high mortality in that species rather than on differences in the causative organism. Mohan and Bhadury (1947) provided a later report of fowl cholera in geese. Van Es and Olney (1940) recognized the marked susceptibility of geese to fowl cholera in using that species to test the infectivity of lots after removal of infected chicken populations.

Birds of prey, waterfowl, and other birds kept in zoological gardens occasionally succumb to infection. Mortalities from fowl cholera in wild geese (Zuydam, 1952), wild ducks (Van den Hurk, 1946; Quortrup et al., 1946), and sea gulls (Kaschula and Truter, 1951) have been reported with spread to domestic fowls. A severe outbreak of fowl cholera in the San Francisco Bay area in 1948 was responsible for a loss of an estimated 40,000 waterfowl (Rosen and Bischoff, 1949).

The disease has been reported in pheasants raised in captivity (Hudson, 1944; Alberts and Graham, 1951), and in captive quail (Hinshaw and Emlen, 1943). Recent reports have recorded the isolation of P. multocida from the flicker (Wickware, 1945) and the starling, grackle, and robin (Bivins, 1953, 1955).

Rabbits are very susceptible to infection with fowl cholera organisms given per os, or by inoculation, and die from septicemic disease. White mice and field mice are also susceptible. Guinea pigs are more resistant but may succumb to intraperitoneal inoculation of P. multocida. By subcutaneous inoculation, a local abscess is produced.

Horses, cattle, sheep, pigs, dogs, and cats are refractory to infection per os, and subcutaneous inoculations result in localized abscesses. All of these animals may succumb, however, to intravenous inoculation of moderately heavy doses. Pasteurella multocida infections, usually of a localized nature, have been encountered in man, although the source of infection has generally not been determined.

Sources of infection. It is often difficult or impossible to determine how fowl cholera is introduced into a flock. Frequently it follows the addition of newly or purchased stock to the breeding flock or the addition of pullets to an older population. Free-flying birds having contact with poultry may provide a source of fowl cholera organisms.

Webster et al. (1927) examined three commercial flocks of White Leghorns for P. multocida and found that many birds harbored the organisms in the nasal clefts. Their presence was related to the amount of fowl cholera and upper respiratory infection in the flocks. They concluded that the endemic focus of infection was "healthy" nasal carriers or "roup cold" cases. In one flock, Pritchett and associates (1930 a,b; 1932) proved that "healthy" chickens saved for breeders were the reservoirs of infection. These had become carriers by having passed through an outbreak of fowl cholera the previous year. From these carriers, the organisms spread and gave rise to acute septicemic disease, localized infections, and the carrier state among contact fowls. The detection and removal of carriers resulted in effective control of the disease in the flock. Van Es and Olney (1940) studied the influence of environment on several poultry diseases and concluded that, in the transmission and dissemination of fowl cholera, apparently healthy birds as infection carriers play a dominant part. Dorsey and Harshfield (1959) reported a higher incidence of fowl cholera outbreaks during late summer and fall months in South Dakota. Carrier birds among the older flock, held over for a second year, provided a reservoir of infection for young susceptible pullets housed with them.

The body excretions of diseased birds which contaminate soil, food, or water can be an important factor in dissemination of the disease. There is evidence that nasal

excretions may have a more dominant role than feces in this regard. Contaminated crates, feed bags, or any equipment used previously for poultry may serve in introducing fowl cholera into a flock. The carcasses of fowls dead of cholera are thoroughly permeated with organisms and may serve as a source of infection, especially because of the tendency of fowls to consume such material. Reis (1931) recovered *P. multocida* from carcasses decomposed in air and in a bird exhumed eleven days after death. Hendrickson and Hilbert (1932) found the organism to remain viable for at least two months in a carcass kept in an ice box. The feeding of offal of dressed poultry is not without danger.

The possibility that insects may serve as vectors of fowl cholera has been considered. Skidmore (1932) experimentally transmitted fowl cholera to turkeys by feeding flies that had previously fed on infected blood, and cautioned that under natural conditions, ingestion of flies might be a means of introducing the disease into a flock. That such transmission is probably not common is borne out in the studies of Van Es and Olney (1910). Although virulent outbreaks of fowl cholera were maintained in two lots of chickens during the height of the fly season, there was no spread of the disease to adjoining lots separated only by poultry netting.

Natural infection and pathogenicity. Webster et al. (1927) administered stock and freshly isolated strains of *P. multocida* per os and intranasally to young and adult chickens, producing infections only in the birds exposed intranasally. They proposed that *P. multocida* infection is primarily a respiratory disease. Hughes and Pritchett (1930) again demonstrated the respiratory route of infection, with negative results by way of the alimentary canal. The response when the organisms were introduced intranasally was varied, some chickens developing acute septicemic cholera and others developing localized infections such as rhinitis and wattle involvement. Still others became healthy carriers. Webster (1930) observed that the severe epi-

demic form of fowl cholera was associated with a relatively virulent type of organism which survives with difficulty in the host, whereas the endemic disease is associated with strains of relatively low virulence and high vegetative capacity.

Cernaianu (1912) considered the fowl cholera organism a facultative pathogen, varying in virulence under different conditions of infection. He attributed the different pathological manifestations seen in fowl cholera to variation in virulence. He concluded that in order for an outbreak to occur, there must be some secondary factors such as season, weather, or nutrition.

Inadequate ventilation has also been suggested as an important contributing factor to the spread and severity of fowl cholera outbreaks. Trillat (1931) observed that mice or chickens placed in closed cages containing small quantities of culture of *P. multocida* were readily infected, provided the enclosed air was humid and contained gaseous products of respiration or solid particles. The actual number of organisms in the air required to produce disease was less than the number required for subcutaneous injection. He stated that transmission from infected to normal animals occurred in cages where there was no contact other than by air.

Pasteur (1880b) had reported that the fowl cholera organism produced toxins in culture media, and it was believed that a negative chemotaxis occurred because of this, allowing for a rapid increase of the organisms in the body. Later works of Weil (1905) and Hadley (1918) did not verify this. It is now recognized that *P. multocida* does not produce an exotoxin.

Symptoms and course of the disease. Van Es and Olney (1910) state that 4 to 9 days usually elapse before appearance of symptoms in a flock naturally exposed to fowl cholera. However, in their experimental flocks, explosive outbreaks sometimes occurred within 48 hours after the introduction of infected birds. In outbreaks of the peracute disease, premonitory symptoms may be entirely lacking, and fowls in good flesh will be found dead on the nest

FIG. 11.2 – Localized Pasteurella infection in wattle.

or beneath the perches. Similar sudden losses occur during the next few days. The rapidly mounting mortality in such flocks is good presumptive evidence of fowl cholera, especially if there are other fowl species on the premises also affected. Cyanosis of the comb and wattles is a frequent but not an invariable symptom.

Later in the progress of the outbreak, sick birds may be found which are listless, refusing to eat or drink, and staying apart from the rest of the flock on the roost, nest,

or floor. These may live for a few hours or linger for several days. Some birds living for a longer period may develop rales and a thick catarrhal nasal discharge. Diarrhea may or may not occur.

Chronic manifestations of Pasteurella infection may occur in flocks following early acute stages or in outbreaks of apparently low virulence. Localization of the organism in the wattles is common in breeds with large pendulous wattles, causing an edematous swelling, later to become caseous (Fig. 11.2). Localization in joints and tendon sheaths of the legs or wings is also common, resulting in swelling and lameness (Fig. 11.3). A few develop torticollis due to localization of P. multocida in the ear or at the base of the skull (Fig. 11.4). Outbreaks which are associated with the more chronic symptoms are prone to continue over a period of several weeks or even months.

The actual death loss in natural outbreaks of fowl cholera varies from a few birds, if brought under control promptly, to 60 per cent or more in outbreaks either of an extremely acute nature or when prolonged as a chronic disease. In chicken flocks, there are some birds which appear to resist infection throughout the outbreak. At least some of these become "nasal carriers" of the fowl cholera organism.

While the symptoms as indicated are highly suggestive of fowl cholera, confirmatory evidence is possible only by necropsy and bacteriological examination with re-

FIG. 11.3 – Localized Pasteurella infection in joint.
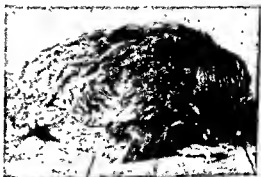
FIG. 11.4 – Torticollis resulting from P. multocida infection in ear.

covery of *P. multocida* from the blood or tissues.

Lesions. The lesions found on necropsy of birds dying of fowl cholera are not constant, but the pathological changes often provide suggestive evidence of the disease. In those which are found dead or died within a few hours after appearance of symptoms, petechiae and ecchymoses involving serous surfaces and the fat of the abdomen are commonly present. Hemorrhages are especially prevalent on the heart surface and gizzard (Fig. 11.5). Similar small hemorrhages may be seen in the lungs and mucosa of the intestine. Marked congestion of the duodenal portion of the intestine with a thick, viscid mucous accumulation in the lumen is a frequent postmortem finding in acute infections. In laying flocks, cheesy material from ruptured yolk sacs may be distributed in the body cavity.

The liver in acute cases reveals a parenchymatous hepatitis. The organ is somewhat swollen, of brownish or yellowish-brown color, and more friable than normal. Numerous pinpoint to pinhead size necrotic foci may be visible on the surface and distributed throughout the liver (Fig. 11.6). In infected birds which survive for several days, the liver may present a greenish color, but enlargement and necrotic foci are less frequently seen. The spleen seldom shows significant change.

In localized infections associated with the more chronic manifestations of fowl cholera, the postmortem findings are variable. Where respiratory symptoms have been predominant, a catarrhal exudate is present in the nasal passages and trachea, and solidification of parts of one or both lungs is evident in some cases. Localization in the wattles causes, at first, an edematous swelling which later is found to be a caseous exudate between the skin folds. In like manner, the exudate in tendon sheaths and joints in Pasteurella localizations may consist of a cloudy fluid or caseous exudate

FIG. 11.5 — Hemorrhages on heart, gizzard, and proventriculus in fowl cholera. (Biester, Iowa State University.)

depending on the duration of the infection. In those birds which have shown "wryneck," a caseous exudate is found in the auditory canal or involving bones at the base of the brain.

Pasteurella multocida occasionally produces localized lesions in other tissues. Thorp *et al.* (1931) observed abscesses in the oviduct, suppurative arthritis of the coxo-femoral articulation, and osteomyelitis of the proximal end of the femur in a group of spontaneously infected birds. Van Es and Olney (1940) recovered *P. multocida* from a necrotic area in abdominal fat, from a caseous plug in the ceca, and from exudate present with an infection of the oviduct.

Diagnosis. Although a presumptive diagnosis may be made from symptoms and lesions, fowl cholera must be differentiated from certain other poultry diseases,

notably fowl typhoid, fowl plague, avian monocytosis, other respiratory infections, synovitis, and localized infections from other causes. Organisms showing bipolar staining characteristics can often be demonstrated in slides smeared with heart blood or liver tissue from birds dead of acute fowl cholera. A conclusive diagnosis, however, is based on cultural methods and the identification of *P. multocida* by its biochemical characteristics.

Immunity. Pasteur (1880b), using a virulent culture of the fowl cholera organism attenuated by prolonged growth on artificial media, produced a vaccine which protected fowls against subsequent exposure. In field use, his method did not prove practical because uniform attenuation could not be secured and heavy losses sometimes occurred in vaccinated flocks. Since Pasteur's classical work, there have
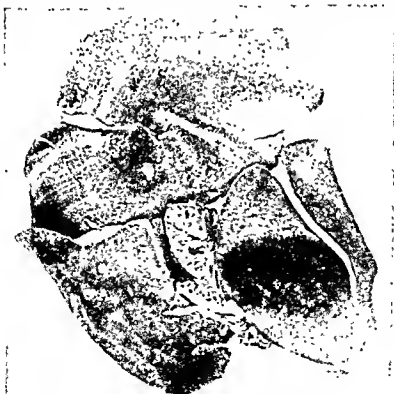
FIG. 11.6 — Foci of necrosis of liver in fowl cholera. *Pasteurella* found in lesions. (Biester, Iowa State University.)

been numerous attempts to produce immunizing agents, but results have been contradictory.

Rodriquez and Antonio (1946) reviewed the literature on artificial immunization against fowl cholera and conducted immunization trials duplicating methods and procedures previously employed and commonly resorted to in practice. They used monovalent and mixed bacterins (formalin-, phenol-, and heat-killed), iodized bacterin, glycoside-lipid, natural aggressins, bacterins of soluble products of P. multocida, formalinized monovalent bacterin precipitated with sodium sulfate, and fowl cholera antiserum. None of these agents gave satisfactory immunity against fowl cholera.

Dougherty (1953) compared chemically killed broth culture bacterins with a chicken embryo culture vaccine (formalin-inactivated) in ducks. Eighty-three per cent of the ducks survived challenge with virulent culture at four weeks in the embryo vaccine group as against 17 per cent in the broth bacterin groups. By the sixth week a marked loss of immunity had occurred. Nelson (1955) obtained inconsistent results in preventing recurrence of losses from fowl cholera with autogenous bacterins in turkey flocks in field outbreaks. Heddleston and Hall (1958) conducted fowl cholera immunization trials comparing a commercial and several experimentally prepared bacterins. A water-in-oil emulsified P. multocida bacterin provided protection against challenge infection for at least 9 months and was superior to any of the other preparations used. In another experiment, a high degree of immunity was established in both chickens and turkeys with the emulsified type bacterin (Heddleston and Reisinger, 1959). An aluminum hydroxide adsorbed bacterin also produced an effective immunity for at least a year in chickens (Heddleston and Reisinger, 1960). In further studies, Heddleston (1962) noted that a P. multocida strain recovered from a fowl cholera outbreak in previously vaccinated turkeys

differed serologically and immunologically from the strain used in the preparation of the bacterin. A bivalent emulsified bacterin containing the two types (serotypes 1 and 3, Little and Lyon classification; serotypes 5:A and 8:A, Namioka and Bruner classification) stimulated and maintained a high level of immunity against both types. A bivalent aluminum hydroxide adsorbed bacterin was less effective.

Boyer and Brown (1963) were not as successful in demonstrating satisfactory immunity in turkeys following vaccination. Although bivalent commercial emulsified bacterins provided better results than monovalent bacterins, over one-third of the vaccinated birds died following challenge with a Little and Lyon type 3 culture.

Dorsey (1963b) selected strains for preparation of emulsified type bacterins from his biochemic Groups I and II. In immunization trials in chickens and turkeys monovalent bacterins provided protection at a high level against challenge with a strain of the same group. Adequate immunity was not demonstrated against challenge with a strain from the other group. However, in one trial in turkeys, the opposite was true. A Group II bacterin failed to protect against challenge by a homologous strain but good protection was provided by the Group I bacterin. He states that biochemic grouping would be useful only for roughly selecting strains for more specific serologic and immunologic identification.

Treatment. Several of the sulfonamides have been employed in treatment of fowl cholera, both experimentally and in natural outbreaks, with varying success. Sulfamethazine, sulfamerazine, and sulfaquinoxaline have received more attention than others of this group of chemotherapeutic agents. Kiser et al. (1948) reported 63 to 85 per cent reduction in mortality in experimentally produced fowl cholera compared to untreated controls, using sulfamethazine and sodium sulfamethazine. In natural outbreaks the reduction of mortality was 45 to 75 per cent. Five-tenths to

compared to 12 per cent in a group which received mash containing oxytetracycline at the level of 500 grams per ton. In six natural outbreaks, oxytetracycline at this level in the feed resulted in checking mortality, but losses recurred in three of the flocks after withdrawal of the antibiotic. In another limited trial a commercially prepared injectable form of oxytetracycline protected birds against a challenge inoculation of *P. multocida* that killed 70 per cent of a similar control group.

Prevention and control. Improved immunological agents can be valuable aids in prevention and control of fowl cholera. Recent developments in delineation of serotypes of *P. multocida* and in methods of preparation of fowl cholera bacterins are encouraging in this respect. However, good management practices must continue to be emphasized. Heddleston and Reisinger (1960) demonstrated that stresses caused by changing the social or peck order of vaccinated males and fowl pox infection in the birds at the time of vaccination and exposure significantly reduced the efficacy of vaccination.

In a management program aimed at prevention of fowl cholera, consideration must be given to the many ways that infection might be introduced and the part that carrier birds play in the dissemination of the disease. Introduction of new birds should be only as chicks and these raised in a clean environment completely isolated from mature fowls. The isolation should be continued in the housing; unless separate houses can be provided for first and second year laying flocks, the older flock should be marketed in its entirety. The separation of different species of fowls on the same premises is likewise important.

The danger of infection being introduced by cockerels or pullets acquired from other flocks or by the return of exhibition birds from fairs or poultry shows should be emphasized. Chicken crates and other pieces of equipment which have been previously used for poultry may serve as sources of infection unless they have received thorough cleaning and disinfection. The fact that *P. multocida* has been recovered from many species of free-flying birds warrants consideration of this source of infection to poultry with measures to prevent their association with the flock.

The application of strict sanitary measures both in the prevention and control of outbreaks is of utmost importance. Particular attention must be given to providing and maintaining clean litter and types of equipment for feed and water which minimize contamination with body excretions.

A practical means for the detection of carriers of the fowl cholera organism has not been developed. Shook and Bunyea (1939) reported the control of an outbreak of fowl cholera due to a carrier condition of several years' standing by means of a stained antigen, rapid whole blood agglutination test of the flock, and the removal of reactors. The stained antigen test agreed with the tube agglutination test on 133 reactor birds and negative controls. Nobrega and Bueno (1944) found that the agglutination test, performed in accordance with the technique of Shook and Bunyea, failed to reveal efficiently the fowl cholera carriers, which were identified by inoculation of pigeons and mice with mouth mucus from individuals of a flock. Dorsey and Harshfield (1959) modified the technique of preparation of an antigen for the rapid whole blood agglutination test but found poor correlation between reactions with antigens of biochemic Group I and Group II strains and isolations of *P. multocida* by nasal swabs or at necropsy in an infected flock of nearly 100 birds.

REFERENCES

Alberts, J. O.: 1950. The prophylactic and therapeutic properties of sulfamerazine in fowl cholera. Am. Jour. Vet. Res. 11-414.
——— and Graham, R.: 1948. Sulfamerazine in the treatment of fowl cholera in turkeys. Am. Jour. Vet. Res. 9.310.
——— and Graham, R.: 1951. An observation on auremycin therapy of fowl cholera in pheasants. Vet. Med. 46 505.

Bierer, B. W.: 1962. Treatment of avian pasteurellosis with injectable antibiotics. Jour. Am. Vet. Med. Assn. 141:1344.

Bivins, J. A.: 1953. Pasteurellosis in a starling. Cornell Vet. 43:241.

———. 1955. Pasteurellosis in feral birds. Cornell Vet. 45:180.

Boyer, C. I., Jr., and Brown, J. A.: 1963. Protection of chickens vaccinated with fowl cholera bacterins. Avian Dis. 7:165.

Carter, G. R.: 1955. Studies on *Pasteurella multocida*. I. A hemagglutination test for the identification of serological types. Am. Jour. Vet. Res. 16:481.

———: 1957. Studies on *Pasteurella multocida*. II. Identification of antigenic characteristics and colonial variants. Am. Jour. Vet. Res. 18,210.

Cernaianu, C.: 1942. Ueber die Geflugelcholera, eine durch Haltung, Ernährung und Ausbeutung bedingte Krankheit. Zeitschr. Infekt-Krankh parasitäre Krankh. u Hyg. Haustiere 58:142.

Curtice, C.: 1902. Goose septicemia. R. I. Agr. Exper. Sta., Bul. 86, p 191.

Delaplane, J. P.: 1945. Sulfaquinoxaline in preventing upper respiratory infection of chickens inoculated with infective field material containing *Pasteurella avicida*. Am. Jour. Vet. Res. 6:207.

Dorsey, T. A.: 1963a. Studies on fowl cholera. I. A biochemic study of avian *Pasteurella multocida* strains. Avian Dis. 7:386.

———: 1963b. Studies on fowl cholera. II. The correlation between biochemic classification and the serologic and immunologic nature of avian *Pasteurella multocida* strains. Avian Dis. 7.393.

———, and Harshfield, G. S.: 1959. Studies on fowl cholera. S. Dak. Agr. Exper. Sta. Tech. Bul. 23.

Dougherty, E., 3rd.: 1953. The efficacy of several immunizing agents for the control of fowl cholera in the White Pekin duck. Cornell Vet. 43:421.

Eveleth, D. F., Goldsby, A. I., Bolin, F. M., Edhlund, N., and Rheault, P.: 1951. Poultry diseases of North Dakota. N. Dak. Agr. Exper. Sta. Bul. 366, p. 12.

Gärtner, A.: 1898. Über das Absterben von Krankheitserregern im Mist und Kompost. Zeitschr. f. Hyg. 28:1.

Gibbs, C. S: 1940. Duck septicemia. Jour. Am. Vet. Med. Assn. 96:239.

Gray, H.: 1913. Avian Cholera. *In* A System of Veterinary Medicine. Vol. 1, p. 420, E. W. Hoare, editor. Alexander Eger, Chicago.

Greenham, L. W., and Hill, T. J.: 1962. Observations on an avian strain of *Pasteurella hemolytica*. Vet. Record 74:861.

Hadley, P. B.: 1918. Studies on fowl cholera. V. Toxins of B. avisepticus. Jour. Bact. 3:277

Hagan, W. A., and Bruner, D. W.: 1961. Infectious Diseases of Domestic Animals, 4th edition, Comstock Publ. Assoc, Ithaca, N.Y., p. 242.

Hall, W. J., Heddleston, K. L. Legenhausen, D. H., and Hughes, R. W.: 1955. Studies on pasteurellosis. I. A new species of *Pasteurella* encountered in chronic fowl cholera. Am. Jour. Vet. Res. 16.598.

Harbourne, J. F.: 1962. A hemolytic cocco-bacillus recovered from poultry. Vet. Record 74.566.

Harry, E. G.: 1962. A hemolytic cocco-bacillus recovered from poultry. Vet. Record 74:640.

Heddleston, K. L.: 1962. Studies on pasteurellosis. V. Two immunogenic types of *Pasteurella multocida* associated with fowl cholera. Avian Dis 6.315.

———, and Hall, W. J.: 1958. Studies on pasteurellosis. II. Comparative efficiency of killed vaccines against fowl cholera in chickens. Avian Dis. 2.322.

———, and Reisinger, R. C.: 1959. Studies on pasteurellosis. III. Control of experimental fowl cholera in chickens and turkeys with an emulsified killed vaccine. Avian Dis. 3: 397.

———, and Reisinger, R. C.: 1960. Studies on pasteurellosis. IV. Killed fowl cholera vaccine adsorbed on aluminum hydroxide. Avian Dis. 4:429.

Hendrickson, J. M., and Hilbert, K. F.: 1932. The persistence of P. avicida in the blood and organs of fowls with spontaneous fowl cholera. Jour. Infect. Dis. 50.89.

Higgins, C. H.: 1898. Notes upon an epidemic of fowl cholera and upon the comparative production of acid by allied bacteria. Jour. Exper. Med. 3:661.

Hinshaw, W. R., and Emlen, J. T.: 1943. Pasteurellosis in California Valley quail. Cornell Vet. 33:351.

Hudson, C. B.: 1944. Fowl cholera in ring-necked pheasants. Jour. Am. Vet. Med. Assn. 104.211.

Hughes, T. P.: 1930. The epidemiology of fowl cholera. II. Biological properties of P. avicida. Jour. Exper. Med. 51:225.

———, and Pritchett, J. W.: 1930. The epidemiology of fowl cholera. III. Portal of entry of P. avicida; reaction of the host. Jour. Exper. Med. 51:239.

Iyer, S. G., and Hashmi, Z. A.: 1945. The occurrence and spread of fowl cholera in India. Indian Jour. Ver. Sci. and Anim. Husb. 15:157.

Kaschula, V. R., and Truter, D. E.: 1951. Fowl cholera in sea-gulls on Dassen Island. Jour. South Africa Vet. Med. Assn. 22.191.

Kiser, J. S., Prier, J., Bottorff, C. A., and Greene, L. M.: 1948. Treatment of experimental and naturally occurring fowl cholera with sulfamethazine. Poultry Sci. 27.257.

Kitt, Th.: 1888. Beitrage zur Kenntnius der Geflügelcholera und deren Schutzimpfung. Deutsch Zeitschr. f. Tiermed. 13:1.

Little, P. A.: 1948. Use of aureomycin in some experimental infections in animals. Ann. N.Y. Acad. Sci. 51:246.

———, and Lyon, B. M.: 1943. Demonstration of serological types within the nonhemolytic *Pasteurellae*. Am. Jour. Vet. Res. 4:110.

McNeil, E., and Hinshaw, W. R.: 1948. The effect of streptomycin on *Pasteurella multocida in vitro*, and on fowl cholera in turkeys. Cornell Vet. 38:259.

Manninger, R.: 1929. Geflugelcholera. *In* Handbuch der Pathogenen Mikroorganismen (Kolle und Wasserman) 6(1):529-62.

Marthedal, H. E., and Velling, G.: 1954. Pasteurellose og pseudotuberkulose hos fjerkrae i Danmark. Nord. Vet. Med. 6:651.

Merchant, I. A., and Packer, R. A.: 1961. Veterinary Bacteriology and Virology. 6th edition, Iowa State University Press, Ames, p 413.

Mohan, R. N., and Bhadury, S. K.: 1947. Some observations on pasteurellosis. I. An outbreak of pasteurellosis in geese. Indian Jour. Vet. Sci. 17:247.

Moore, V. A.: 1916. Pathology and Differential Diagnosis of the Infectious Diseases of Animals. 4th edition, Macmillan Co., New York, p. 74.

Namioka, S., and Bruner, D. W.: 1963. Serological studies on *Pasteurella multocida*. IV. Type distribution of the organisms on the basis of their capsule and O groups. Cornell Vet. 53:41.

———, and Murata, M.: 1961a. Serological studies on *Pasteurella multocida*. I. A simplified method for capsule typing the organism. Cornell Vet. 51:498.

———, and Murata, M.: 1961b. Serological studies on *Pasteurella multocida*. II. Characteristics of somatic (O) antigen of the organism. Cornell Vet. 51:507.

———, and Murata, M.: 1961c. Serological studies on *Pasteurella multocida*. III. O antigenic analysis of cultures isolated from various animals. Cornell Vet. 51:522.

Nelson, C. L.: 1953. The veterinarian in poultry practice. Proc. 92nd Ann. Meet. Am. Vet. Med. Assn. p 506.

Nobrega, R., and Bueno, R. C.: 1944. On the detection of fowl cholera carriers. Arq. Inst. Biol. São Paulo. 15:339.

———: 1950. The influence of the temperature on the viability and virulence of *Pasteurella avicida*. Bol. da. Soc. Paulista de Med. Vet. 8.189.

Pasteur, L.: 1880a. Sur les maladies virulents et en particulier sur la maladie appelée vulgairement choléra des poules. Compt. Rend. Acad. Sci. 90 239, 952, 1030.

———: 1880b. De l'atténuation du virus du choléra des poules. Compt. Rend. Acad. Sci. 91.673.

Peterson, E. H.: 1948. Sulfonamides in the prophylaxis of experimental fowl cholera. Jour. Am. Vet. Med. Assn. 113:203.

Pritchett, I. W., Beaudette, F. R., and Hughes, T. P.: 1930a. The epidemiology of fowl cholera. IV. Field observations of the "spontaneous" disease. Jour. Exper. Med. 51:249.

———, Beaudette, F. R., and Hughes, T. P.: 1930b. The epidemiology of fowl cholera. V. Further field observations of the spontaneous disease. Jour. Exper. Med. 51:259.

———, and Hughes, T. P.: 1932. The epidemiology of fowl cholera. VI. The spread of epidemic and endemic strains of *Pasteurella avicida* in laboratory populations of normal fowl. Jour. Exper. Med. 55:71.

Quortrup, E. R., Queen, F. B., and Merovka, L. J.: 1946. An outbreak of pasteurellosis in wild ducks. Jour. Am. Vet. Med. Assn. 108.94.

Reis, J.: 1931. Estudos sobre cholera aviaria I. Diagnostico de cholera em cadaveres em putrefacção. Arch. Inst. Biol. Defesa. Agric e Animal 4.291.

Roberts, R. S.: 1947. An immunological study of *Pasteurella septica*. Jour. Comp. Path. and Therap. 57:261.

Rodriquez, L., and Antonio, J.: 1946. Immunidad contra el colera avario. Gaceta Veterinaria 8:66.

Rosen, M. N., and Bischoff, A. I.: 1949. The 1948-49 outbreak of fowl cholera in birds in the San Francisco bay area and surrounding counties Calif. Fish and Game 35:185.

Rosenbusch, C., and Merchant, I. A.: 1939. A study of the hemorrhagic septicemia Pasteurellae. Jour. Bact. 37:69.

Salmon, D. E.: 1880. Investigations of fowl cholera. Rep. U.S. Comm. Agr. p 401.

———: 1881-82. Investigations of fowl cholera. Rep. U.S. Comm. Agr. p. 272.

———: 1883. Investigations of fowl cholera. Rep. U.S. Comm. Agr. p 44.

Shook, W. B., and Bunyea, H.: 1939. The detection of carriers of fowl cholera and its control by means of a stained-antigen rapid whole-blood test. Poultry Sci. 18:146.

Skidmore, L. V.: 1932. The transmission of fowl cholera to turkeys by the common house fly (*Musca domestica* Linn) with brief notes on the viability of fowl cholera microorganisms. Cornell Vet. 22:281.

Thorp, F. Jr., James, W. A., and Graham, R.: 1931. An unusual form of fowl cholera. No. Am. Vet. 12(2):37.

Trillat, A.: 1931. Experience d'infection par voie aérienne. Cas du choléra des poules. Compt. Rend. Acad. Sci. 192:1598.

Van den Hurk, C.F.G.W.: 1946. Aantekenungen bij de epizootie van vogelcholera over Nederland in het najaar van 1945. Tijdschr. voor Diergeneesk. 71:361.

Van Es, L., and Olney, J. F.: 1940. Inquiry into the influence of environment on the incidence of poultry diseases. Nebr. Agr. Exper. Sta., Res. Bul. 118, p. 17.

Ward, A. R., and Gallagher, B. A.: 1920. Diseases of Domesticated Birds. Macmillan Co., New York, p. 242.

Webster, L. T.: 1930. The epidemiology of fowl cholera. Experimental studies. Jour. Exper. Med. 51:219.

————, Hughes, T. P., Pritchett, I. W., and Beaudette, F. R.: 1927. *Pasteurella avisepticum* infection in poultry. Proc. Soc. Exper. Biol. and Med. 25:119.

Weil, E.: 1905. Untersuchungen über Infektion und Immunität bei Hühnercholera. Arch. f. Hyg. 52:412.

Wickware, A. B.: 1945. Case reports of relatively infrequent diseases observed at the poultry pathology laboratory. Canad. Jour. Comp. Med. 9:151.

Wooldridge, W. R.: 1954. Farm Animals in Health and Disease. Crosby Lockwood and Son, Ltd., London, p. 428.

Zuydam, D. M.: 1952. Penicilline als therapeuticum bij vogelcholera. Tijdschr. voor Diergeneesk. 77: 256.

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12

Tuberculosis of Poultry

Tuberculosis of poultry may be defined as a contagious disease caused by *Mycobacterium avium*. The disease is characterized by its insidious chronicity, its long continuation in a flock when once established, and its tendency to induce in infected birds a state of unthriftiness, decrease or stoppage of egg production, and finally death.

The announcement that tuberculosis of man and of cattle was due to a living bacterial parasite – the tubercle bacillus – was made by Koch in 1882. Tuberculosis of chickens was first recognized as a related but separate entity by Cornil and Mégnin (1884). Whether or not the microorganism of tuberculosis of chickens is identical with the microorganism responsible for tuberculosis of mammals provided much controversy. Koch (1890) maintained for many years that tubercle bacilli were always the same regardless of the species in which they might occur. However, Rivolta, and later Maffucci (1890), showed by con-

vincing experimental procedures that the microorganism of tuberculosis of chickens is definitely dissimilar to that of bovine tuberculosis. In 1901 Koch[1] finally abandoned his previous position and declared that tuberculosis of poultry is unlike tuberculosis of human beings and that the disease in man is dissimilar to that of cattle. Consequently, it was settled finally that three different species of tubercle bacilli are concerned with tuberculosis of mammals and fowl. Differences in the three species, which can be recognized readily at the present time, were vague and confused for nearly twenty years after Koch had demonstrated that a specific microorganism is the cause of tuberculosis.

Although tuberculosis of chickens had been recognized as a contagious disease even before Koch succeeded in demonstrating the tubercle bacillus, the disease has continued to spread throughout most

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[1] Address published 1902 (Koch, 1902).

[374]

of the civilized world. With the available information on the nature of the disease, its eradication in the United States is entirely feasible. To fail to suppress the malady perpetuates an entirely unnecessary economic burden on American agriculture.

The more important reasons why tuberculosis of poultry should be eliminated may be stated briefly as follows: (1) affected birds are unthrifty; (2) tuberculous chickens are undesirable for human food; (3) diseased birds produce fewer eggs; (4) tuberculous chickens frequently are the source of tuberculosis of sheep and especially swine; and (5) avian tubercle bacilli are capable of sensitizing cattle to mammalian tuberculin.

The indictment against the tuberculous chicken is serious, and nothing has been offered to refute the fact that a tuberculous bird is an undesirable member of the farm economy and as such should be

eliminated. Unfortunately, in the United States the presence of tuberculosis of chickens continues to be accepted with a certain complacency, and we have failed as yet to formulate a comprehensive program that can be expected to eradicate the disease. The noteworthy results obtained in the program for the eradication of bovine tuberculosis should stimulate the attack on the disease in chickens.

INCIDENCE AND GEOGRAPHIC DISTRIBUTION

Tuberculosis of chickens is world-wide in its distribution; however, the disease occurs more frequently in the North Temperate Zone than elsewhere. Although the disease exists in practically all of the United States, there is a marked difference in its incidence in different parts of the country. The highest incidence of infection occurs in flocks of the western and eastern North Central States (Fig. 12.1). The states

MATURE CHICKENS INSPECTED
And Number Condemned for Tuberculosis

% Condemned

N. Atlantic ...... .006
S. Atlantic .... .001
S. Central .... .004
E. N. Central 3
W. N. Central ..... .79
Western .... .003

Calendar year 1962

U.S. DEPARTMENT OF AGRICULTURE
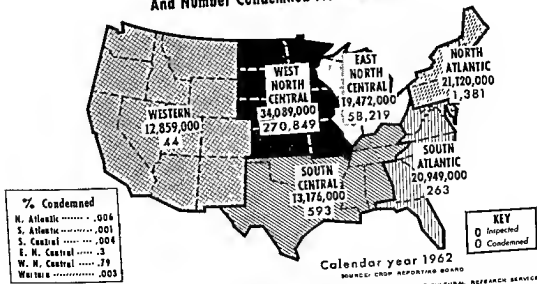
FIG. 12.1 — Relative incidence of tuberculosis of poultry in the United States based on data compiled by the Agricultural Research Service, USDA. Note that the highest percentage of condemnations for tuberculosis for the year 1962 was among chickens in six states, comprising the West North Central area, the lowest percentage of condemnations was in chickens in the 11 states designated "Western." (Courtesy of Dr. A. F. Ranney, Chief, Tuberculosis Eradication Section, USDA.)

in which the disease is most prevalent include North Dakota, South Dakota, Kansas, Nebraska, Minnesota, Iowa, Missouri, Wisconsin,[2] Illinois, Michigan, Indiana, and Ohio. Although no data are available as to what percentage of individual chickens in the states just mentioned are tuberculous, there are available figures that indicate that in some areas more than 50 per cent of the flocks may be infected. The incidence of the disease in the western and southern states is quite low. The explanation for this is not entirely obvious, although there are several possible contributing factors such as climate, flock management, and duration of the infection. The maintenance of large flocks in the north central part of the United States and the necessity, for climatic reasons, to keep the birds closely confined during winter months provide favorable conditions for the spread and continuation of the disease.

The difficulty of tuberculin-testing all chickens in the United States, or of testing even a majority of the flocks, makes it impossible to obtain exact data on what the incidence of tuberculous infection of chickens really is. However, figures are available as a consequence of various surveys that indicate in a general way the extent of the infection. Information obtained from such sources suggests that the over-all incidence of tuberculosis in poultry for the entire United States is gradually receding and is probably less than 5 per cent.

These figures may be misleading unless it is remembered that the incidence of infection varies greatly in different sections of the country. In the heavily infected western North Central States, where the disease is most prevalent, a conservative estimate would place the percentage of flock infection in some areas as high as 50, whereas in the northern Atlantic States, the southern Atlantic States, the South Cen-

tral States, and the western states, the percentage of infection is negligible (Fig. 12.1). The infection rate varies greatly for individual flocks in the United States, from less than 5 per cent to as much as 95 per cent.

How reliable data may be as indicative of the exact situation regarding the prevalence of avian tuberculosis throughout the entire United States is difficult to appraise. If there has in fact been a significant reduction in the prevalence of the disease, it is probably due in considerable part to the changing concept of poultry husbandry. During the past several years increasing emphasis has been placed on the commercial desirability of young rather than adult birds. However, it is not likely that the prevalence of the disease has changed appreciably among long-established farm flocks where poultry is maintained more or less incidental to general farming operations.

As in the United States, the incidence of tuberculosis of chickens varies greatly in the different areas of Canada. From available data the incidence of infection in the different provinces varies from 1 per cent to 26 per cent. The disease occurs to only a limited extent in the Panama Canal Zone and has been observed but infrequently in Chile, Cuba, and Puerto Rico. The disease has not been reported from Colombia.

Considerable information concerning the incidence of avian tuberculosis in Europe is available. In Bulgaria the incidence of the disease is relatively low. The disease is rather prevalent in England and to probably a less extent in Scotland. Adequate information regarding the incidence of the disease in France is not at hand. However, the disease does exist in that country. Data from German sources prior to World War II indicate that avian tuberculosis was extremely prevalent in the German Reich and that the disease constituted a serious handicap to the poultry industry of that country. Tuberculosis of chickens is practically unknown in Greece, infrequent in Switzerland, and apparently of rare oc-

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[2] According to Hastings and Halpin (1913), tuberculosis was not recognized by the Agricultural Experiment Station of Wisconsin as occurring in poultry flocks of that state prior to 1906

currence in Italy. The disease occurs in Spain and seems to have assumed serious proportions in certain of the Baltic States. Avian tuberculosis is of frequent occurrence in certain districts of Norway. The disease occurs in Holland and is apparently on the increase in Czechoslovakia and Bessarabia.

Avian tuberculosis has been observed in a few instances in Indonesia, but it is apparently rare or nonexistent in the Philippines and in the state of Israel.[2] The disease has been noted in the Union of South Africa and in New Zealand. In China there is a question whether the disease exists at all, although in Mukden there is evidence that the infection is fairly common.[4]

Age in relation to incidence. Aside from the influence of climate and the factors of environment, the incidence of infection also depends upon the age of the chickens. This is well illustrated by the following data.[5] Twenty-eight farm poultry flocks in one county in Illinois were tuberculin-tested, and fourteen, or 50 per cent of the flocks, were found to contain tuberculous birds. In these flocks the incidence of tuberculosis among a total of 1,476 hens more than one year of age was 11.1 per cent. In 1,056 pullets (less than one year of age) in the same flocks, the incidence of infection as determined by the tuberculin test was 0.19 per cent. Hays (1929) found among 40,073 chickens tested in Nebraska that the incidence of tuberculosis was 9.3 per cent. When the tuberculous birds were considered in relation to the different age groups, it was found that 77.6 per cent of the infected chickens were more than one year of age and 22.4 per cent were one year of age or less.

These data confirm the view that tuberculosis becomes more prevalent as the ages of the birds advance. There is evidence that indicates, however, that infection is less likely to succeed if exposure is delayed until the chickens reach the state of maturity, and that the disease when present in adult birds represents in most instances a process that began months or even years before, when the animal was young. Tuberculosis appears to be less prevalent in young fowl than in older ones, not because the younger are more resistant to infection than the older, but because in the older birds the disease has had a greater opportunity to become established as a consequence of a longer period of exposure.

Although the lesions of tuberculosis in young chickens are usually less severe than the lesions in the adult, extensive or generalized tuberculosis in young chickens has been observed occasionally. Such an animal obviously constitutes an important source of dissemination of virulent tubercle bacilli and must be considered a menace to other fowl and to susceptible mammals. That generalization of the disease does occur in young birds provides a formidable argument against the claim that avian tuberculosis can be eliminated by disposing of the older hens and maintaining a flock of young birds only. It is true that the concentration of potentially infective materials will be diminished eventually if only young birds constitute the flock, but if the flock is maintained on contaminated premises new infections are likely. The presence of even one pullet that has lesions along the intestinal tract will provide an adequate source of tubercle bacilli to insure the continuance of the disease.

THE CAUSATIVE AGENT[6]

Morphology. The agent responsible for avian tuberculosis is a member of the genus *Mycobacterium* and is known correctly as *Mycobacterium avium*. The most characteristic feature of the organism is its acid-

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[2] According to Dr. J. Van der Hoeden, Director of Veterinary Institute, Tel-Aviv, tuberculosis does not exist among chickens in Israel (personal communication to the author, 1951).

[4] For a more detailed account of the occurrence of tuberculosis in chickens in various parts of the world, consult the monograph by Feldman (1938).

[5] Information supplied many years ago by the late H. R. Smith, Livestock Commissioner, National Livestock Exchange, Chicago.

[6] Methods for the isolation and culture of tubercle bacilli are given in detail in a monograph on avian tuberculosis infections (Feldman, 1938).

fastness.[7] The organism is capable of considerable pleomorphism, this feature being dependent on the character and chemical composition of the medium on which the bacteria are grown. While the organisms are bacillary in character, with perfectly straight forms usually present, clublike, curved, and crooked forms are also usually seen in most preparations. Branching infrequently occurs. Most of the bacteria have rounded ends. The bacteria vary in length from 1 to 3μ, and the average length has been determined to be 2.7μ. Spores are not produced, and the organism is nonmotile.

Reproduction of the avian tubercle bacillus is by simple fission. The existence of a filter-passing form of the avian tubercle bacillus has not been established. Spherical or conical granules occur in the endoplasm. These usually occur anywhere along the length of the bacterium. Whether or not the avian tubercle bacillus is capable of giving rise to forms comparable to Much's granules is uncertain. Although the avian tubercle bacillus will grow and retain its virulence under a variety of atmospheric conditions, the organism is generally considered as aerobic.

The avian tubercle bacillus is not as exacting in its temperature requirements as are the human and the bovine forms of the organism. The avian form of the bacillus will grow at temperatures ranging from 25° to 45° C., although the most

[7] The Ziehl-Neelsen method of staining tubercle bacilli:

1. Cover a fixed film or smear preparation with Ziehl-Neelsen's carbolfuchsin (prepared by adding 10 cc. of a saturated alcoholic solution of basic fuchsin to 90 cc. of a 5 per cent aqueous solution of phenol). Steam gently but continuously for 2 minutes.

2. Wash in water and decolorize with methylene stain by applying an excess of acid alcohol (prepared by adding 2 cc. of hydrochloric acid to 98 cc. of 80 per cent alcohol). Decolorization should be complete in 10 to 20 seconds.

3. Wash in water and counterstain with Löffler's methylene blue for 3 to 5 seconds.

4. Wash in water and dry. Examine with the oil immersion lens.

Tubercle bacilli and other mycobacteria stain bright red; other microorganisms stain blue.

favorable temperature is between 39° and 40° C.

Cultural distinctions. The avian tubercle bacillus is not a difficult organism to cultivate artificially. Stock strains will grow on most solid mediums although for original isolation of the organisms from naturally infected material one of the special mediums designed for culturing tubercle bacilli is desirable. Glycerinated or nonglycerinated mediums are satisfactory, but the resultant colonies are larger if the medium contains glycerin than if it does not. After a culture has been obtained on solid mediums, subcultures usually will succeed in liquid mediums.

On mediums containing whole egg or egg yolk seeded with material prepared from tuberculous tissue and incubated at 37.5° to 40° C., the bacteria usually will become evident in 10 days to 3 weeks as small, slightly raised, discrete, grayish-white colonies (Fig. 12.2). If the inoculum is rich in bacteria, the resultant colonies will be numerous and may tend to coalesce into granulated masses, but there is slight, if any, tendency for the individual colonies to spread. The colonies are hemispherical and do not penetrate into the substance of the medium. If the medium contains glycerin, the colonies gradually change from grayish-white to light ocher. The color becomes darker as the age of the culture increases.

Subcultures on solid mediums show evidence of growth within a few days and reach a maximal development in three to four weeks. Such cultures usually appear moist and unctuous, the surface eventually becoming roughened. The growth is translucent and has a creamy or sticky consistency; it is readily removable from the underlying medium.

Other biochemical characteristics of *M. avium* are: test for niacin is negative, high catalase content, test for peroxidase usually negative, and failure to hydrolyze Tween 80.

In liquid mediums, such as glycerinated broth, growth occurs at the bottom as well

FIG. 12.2 — *Mycobacterium avium*. Primary isolation cultures showing the enhancement of growth in the presence of glycerin (two slants at left) in comparison with growth on nonglycerinated medium (two slants at right).

as at the surface. The surface growth is represented by a delicate filmy pellicle which, as growth continues, becomes thickened, slightly wrinkled, and granular. A "ring" of growth extending upward from the surface often occurs on the inner wall of the flask. Even after prolonged incubation, if the growth is not contaminated, the medium remains clear. The growth finally becomes golden yellow and gives off a characteristic odor.

Although most strains of *Mycobacterium avium* are smooth and moist when first isolated, cultures of avian tubercle bacilli have been noted that were rough, dry, and crumbly. The occurrence of such strains is sufficient reason for caution in designating the type of any tubercle bacillus without tests for pathogenicity.

In vitro, the avian tubercle bacillus is capable of dissociation. By this phenomenon marked variations in the physical character of the colonies become evident. The following variants have been noted: "S" or smooth, which produces a raised, smooth, moist growth; "FS" or flat and smooth, in which the growth is slightly wrinkled and moist and in which the colonies are larger than those of the "S" variant; "R" or rough, in which the colonies are large, dry, and wrinkled; and "CH" or chromogenic. The last-named is physically not unlike

the "S" variant except that the colonies become ocher.

In addition to the physical differences between the variants, there also exist antigenic and pathogenic differences that are significant to a thorough understanding of the variation that occurs after natural infection with the avian tubercle bacillus. Experimentally, it has been noted that the "S" variant is far more pathogenic than the markedly dissimilar "R" variant, whereas the "CH" variant is essentially avirulent.[3]

Stability of types. Although nearly all strains of avian tubercle bacilli possess the essential cultural and pathogenic characteristics necessary to distinguish this organism from the other species of mycobacteria, occasionally strains have been encountered that appear to be atypical. The most likely explanation for the occurrence of such strains is that they represent variants that are themselves unstable. Some contend that the respective types of tubercle bacilli are not necessarily stable and that transmutation of one type into another may occur as a consequence of environmental adaptation. This is a controver-

[a] A more complete discussion of dissociation and colony variation will be found on page 50 of the monograph on avian tuberculosis infections (Feldman, 1938).

sial question, and while it remains unsettled the balance of evidence is strongly on the side of stability.[9]

Antigenic properties. Although the human and the bovine forms of the tubercle bacillus are serologically indistinguishable by either the agglutination or the complement-fixation reaction, the antigenic structure of the avian tubercle bacillus is unlike that of the mammalian forms and may be distinguished from the latter by agglutinin absorption. However, avian tubercle bacilli do not constitute a homogeneous group. There exist at least three avian subtypes that can be distinguished serologically.[10] Distinguishing precipitins have not been demonstrated.

Sensitivity in vitro to certain antimicrobials. The antimicrobial sensitivity of a strain of biologically proven M. avium isolated from a patient affected with pulmonary silicosis was reported by Karlson and associates (1955). It was determined that the microorganisms were resistant to 10 but not to 50 micrograms of streptomycin, to more than 10 micrograms of para-aminosalicylic acid, and to more than 40 micrograms of isoniazid per milliliter of medium, respectively. It was mentioned that the respective sensitivities recorded for the three antimicrobials were characteristic of avian tubercle bacillus.

CHEMISTRY OF THE AVIAN TUBERCLE BACILLUS[11]

The avian tubercle bacillus contains carbohydrates, lipids, and proteins, all of which, according to Sabin (1932), play a part in the cellular reactions of tuberculosis. Some of the carbohydrates are readily extractible; others occur in chemical com-

bination as, for example, the polysaccharides, manninositose, and trehalose, which are esterified with fatty acids. Renfrew (1929) has studied the carbohydrates which may be extracted with water from the defatted cells. The fraction, 1.4 per cent of the defatted cells, which may be precipitated with basic lead acetate, is smaller than the similar fraction from tubercle bacilli of the human type. Chargaff and Moore (1944) have isolated glycogen from avian tubercle bacilli by high-speed centrifugation. Renfrew reported the presence of about 10 per cent nitrogen in the defatted cells, of which 4.4 per cent is in water-soluble protein which possesses the characteristic biological activity of tuberculin, and 34 per cent is in protein, soluble in dilute alkali, with a much larger quantity remaining undissolved. Using another procedure, Menzel and Heidelberger (1938) isolated four fractions of proteins which gave a total of 14.2 per cent protein in the dried defatted cells. By serologic experiments, they demonstrated definite differences in specificity between the protein fractions of the avian and the corresponding protein fractions of the human and bovine types of tubercle bacilli.

The lipids of the tubercle bacilli have been studied extensively by Anderson (1942) and co-workers. With mild methods of extraction they (Anderson et al., 1930a and b) found the lipid content of dried avian tubercle bacilli to be 15.26 per cent; by more vigorous extraction, they (Anderson et al., 1940) obtained an additional 10.8 per cent of firmly bound lipids. The lipids that are extracted by the mild treatment are phosphatides, acetone-soluble fat, and chloroform-soluble wax. The avian tubercle bacillus contains smaller quantities of phosphatides and acetone-soluble fat and a much larger quantity of the firmly bound lipids than does the human type tubercle bacillus.

Like other tuberculophosphatides, those from avian tubercle bacilli contain low percentages of phosphorus and nitrogen (Anderson et al., 1930a and b). The fol-

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[9] Observations on what appeared to be a temporary modification of avian tubercle bacilli in naturally infected crows were reported by Mitchell and Duthie (1950) and in wood pigeons (Columba palumbus) by McDiarmid (1948).

[10] For a detailed account of the antigenic features of M. avium determined serologically, the report by Rotach (1947) may be consulted.

[11] This section was kindly prepared by Dr. Eunice V. Flock, Mayo Graduate School of Medicine, Rochester, Minnesota.

lowing fatty acids are found: palmitic, stearic, oleic, and a new group of saturated liquid fatty acids. The fatty acids are esterified with manninositose instead of glycerol. The saturated liquid fatty acids resemble tuberculostearic acid from the human strain in their biological activity. In the neutral fat, the fatty acids are esterified with trehalose.

The crude chloroform-soluble wax extracted from the tubercle bacillus accounted for 70.7 per cent of the total lipids (Anderson and Roberts, 1930a). The wax contained two optically active hydroxy acids or mycolic acids of high molecular weight which had the property of acid fastness (Anderson and Creighton, 1939). Trehalose and d-eicosanol-2 were also found in the wax. Later two waxes C and D were described. Wax C consists mainly of unusually long chain fatty acids which are esterified with polyhydroxy compounds such as phthiocerol, glycerol, and trehalose to form lipids of relatively high molecular weight, ranging from 1000 to 5000 (Noll, 1957; Miquel et al., 1963). Wax D contains high molecular lipopolysaccharides including 4 mycolic acids, galactose, glucose and arabinose.

Specific glycolipids called mycosides (Smith et al., 1960) have been found in ethanolether extracts of tubercle bacillus. Mycoside C produced by Mycobacterium avium differs from other mycosides in that it contains a peptide. Three different amino acids, D-phenylalanine, allo-threonine, and D-alanine are linked in a pentapeptide. The sequence of amino acids has been determined (Jollès et al., 1961). A mixture of unsaturated hydroxy acids and two molecules of acetic acid are also present in mycoside C. Individual strains of Mycobacterium avium appear to produce mixtures of glycolipids with the same monosaccharide units but it is possible that variation occurs in the proportion and types of monosaccharides from strain to strain (MacLennan, 1962). Eight sugars have been isolated from mycoside C, glucose, arabinose,

rhamnose, 3-0-methylrhamnose, 2,3 and 3,4-0-methylrhamnose, 6-deoxytalose, and 3-0-methyl-6-deoxytalose.

Konno (1956) has shown by a direct qualitative chemical method that M. avium does not synthesize nicotinic acid. A modification of this test by Runyon et al. (1959) is recommended.[12]

SUMMARY OF DISTINGUISHING FEATURES

The more essential features that distinguish avian tubercle bacilli from the human and bovine forms of the organism are summarized in Table 12.1. Although in most instances differences in the physical properties are sufficiently impressive to separate the avian from the mammalian forms of the tubercle bacillus, in critical work in which convincing proof of identification is desirable the animal inoculation method is the procedure of choice. In other words, pathogenic behavior is more important than physical characteristics in revealing whether or not an acidfast organism is the avian, human, or bovine form of the tubercle bacillus.

PATHOGENICITY FOR OTHER FOWL

All species of birds are capable of being infected with avian tubercle bacilli. Some species are more susceptible than others, and generally speaking, those that are domesticated are affected more frequently than those living in a wild or free state. Among the domesticated fowl other than chickens in which tuberculosis may occur are ducks, geese, swans, peacocks, and turkeys. Although tuberculosis occasionally develops in domestic pigeons as a consequence of infection with avian tubercle bacilli, there is evidence that pigeons are more resistant to the infectious agent than are chickens. Parrots and canaries may be

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[12] Those interested in a comprehensive account of the chemical aspects of the growth and metabolism of tubercle bacilli, the chemical features of tuberculosis tissue, and the more important chemotherapeutic agents used in treating tuberculosis in human beings may consult the authoritative treatise by Long (1958).

TABLE 12.1
SUMMARY OF ESSENTIAL DIFFERENCES BETWEEN AVIAN, HUMAN,
AND BOVINE FORMS OF THE TUBERCLE BACILLUS

	Avian	Human	Bovine
Growth in egg medium (without glycerin)	Grows readily. Culture moist and unctuous Optimal temp. 40° C.	Grows well. Culture dry and roughened. Optimal temp. 37.5° C.	Grows slowly. Culture thin and without pigment. Optimal temp. 37.5° C.
Growth in liquid medium	Pellicle formation with crumbly granular growth at bottom	Pellicle formation with growth limited to surface	Pellicle formation with growth limited to surface
Miscibility with saline solution	Suspension easy. Organisms uniformly distributed	Suspension difficult. Organisms form clumps	Suspension difficult. Organisms form clumps
Tuberculin sensitivity	More intense for homologous tuberculin	More intense for mammalian tuberculin	More intense for mammalian tuberculin
Pathogenicity*	Virulent for chickens and rabbits Slightly pathogenic for guinea pigs	Nonpathogenic for chickens. Markedly virulent for guinea pigs but only slightly so for rabbits	Nonpathogenic for chickens. Markedly virulent for guinea pigs and rabbits

* When testing tubercle bacilli for pathogenicity, chickens and rabbits should be inoculated intravenously; guinea pigs, subcutaneously

infected with avian tubercle bacilli, but it is the general impression that these species are less susceptible to avian tubercle bacilli than to the mammalian forms of the organism. Naturally acquired *M. avium* infection in a small flock of Muscovy ducks, and human type tuberculosis in an Amazon parrot, presumably acquired from a tuberculous person, were reported by Hinshaw (1933a and b).

Among wildfowl, tuberculosis is uncommon. However, in wild birds that frequent farm premises where tuberculosis is prevalent in chickens, the disease may be expected to develop. Pheasants *seem to be* markedly susceptible to infection by the avian tubercle bacillus, and the disease has also been observed in the sparrow, the crow,[13] the barn owl, the cowbird, the

blackbird, the eastern sparrow hawk, and the wood pigeon.[14]

Tuberculosis is common among birds in many zoological gardens. In the unnatural environment of captivity, the incidence of the disease frequently equals or even exceeds that for the domestic species of fowl. The infectious agent in practically all instances is the avian tubercle bacillus, although a few instances have been reported of birds being infected with heterologous strains of tubercle bacilli. Tuberculosis in the parrot is usually due to either the human or the bovine type of bacillus.

Some years ago, Ratcliffe (1946) presented convincing evidence that frequency

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[13] Mitchell and Duthie (1950) reported tuberculosis in 9.5 per cent of 263 crows (*Corvus brachyrynchos*). The birds were obtained from western and from northwestern Ontario. Of the 25 infected crows observed, tuberculosis lesions occurred in the livers of 23, in the spleens of 8, in the lungs of 2, and elsewhere in 4.

[14] Some years ago, a mycobacterial, tuberculosis-like disease of the migratory wood pigeon (*Columba palumbus*) was reported from Denmark by Christiansen et al. (1946). Later, what appeared to be a similar condition was reported in England by McDiarmid (1948). The Danish workers failed to transmit the infection experimentally, or to culture the organism artificially, whereas McDiarmid succeeded in culturing the organism and observed a restoration of virulence after serial passage *in vivo*.

TABLE 12.2

COMPARATIVE PATHOGENICITY OF *Mycobacterium avium* FOR CERTAIN MAMMALS

Animal	Susceptibility	Animal	Susceptibility
Cat	Highly resistant	Marsupials	Infection reported
Cattle	Infection occurs; usually localized	Mink	Readily infected
Deer	Infection reported	Monkey	Highly resistant
Dog	Highly resistant	Mouse	Relatively resistant
Goat	Assumed to be relatively resistant	Rabbit	Readily infected
Guinea pig	Relatively resistant	Rat	Relatively resistant
Hamster	Susceptible (intratesticularly)	Sheep	Moderately susceptible
Horse	Assumed to be highly resistant	Swine	Readily infected
Man	Highly resistant		

of incidence of tuberculosis in captive wild birds was related to dietary factors that are remediable. Following the change of diet, to provide adequate protein, vitamin B complex elements, vitamins A and D, and iodized salt, the frequency of the disease in the bird collection of the Philadelphia Zoological Garden was reduced significantly.

PATHOGENICITY FOR MAMMALS[15]

The bacterium responsible for tuberculosis of fowl has a definite pathogenicity for some important species of domesticated mammals and at least a slight pathogenicity for others. This fact should be recognized more widely if the problem of eliminating tuberculosis infections is to be attacked intelligently and eventually solved.

Under conditions of natural exposure it is very exceptional for aggressive, extensive tuberculosis to develop in mammals other than rabbits and swine as a consequence of avian tubercle bacilli. Infection may occur, but the disease remains benign and localized. However, the microorganisms may assume a parasitic existence and multiply in the tissues for a considerable period and, in some instances, may induce a state of sensitivity to tuberculin even though recognizable alterations in the tissues cannot be found (Feldman, 1960). Although spontaneous infection of mammals fails in most instances to produce a disease of comparable severity to that which develops in fowl infected with avian tubercle bacilli, it is possible to produce extensive changes in many species of mammals by introducing the infective agent artificially. The relative pathogenicity of *M. avium* for many of the domesticated mammals is summarized in Table 12.2.

Cattle. Concerning the ability of avian tubercle bacilli to infect cattle, the significant information may be summarized as follows: (1) Avian tubercle bacilli have at least a limited pathogenicity for cattle. (2) The morbid changes produced are inclined to remain localized. Whether or not avian tubercle bacilli are ever responsible

for destructive, widespread, or generalized tuberculosis in cattle is not definitely known.[16] (3) The avian tubercle bacillus is apparently capable of parasitic existence in the tissues of cattle without necessarily giving rise to recognizable tissue changes. However, when lesions occur they show the structural changes ordinarily associated with tuberculosis. (4) Following natural exposure to avian tubercle bacilli, cattle may become sensitized to avian tuberculin, and some of the animals may react to mammalian tuberculin also. (5) The interpretation of the tuberculin test in cattle is made more difficult and uncertain if tuberculous swine or poultry exist on the same premises (Feldman, 1960).

Swine.[17] In an infected environment swine readily become infected with avian tubercle bacilli. Since the beginning of federal supervision of abattoirs in the United States, it has been noted that the incidence of tuberculosis in swine exceeds that in cattle. For example, among approximately 67.1 million swine slaughtered and federally inspected during the fiscal year ending June 30, 1962, parts or the entire carcasses of 1.51 million or 2.25 per cent of the animals were found to be tuberculous. This is in marked contrast to the incidence of tuberculosis in cattle. During the same period of time, the carcasses of approximately 20.1 million cattle were examined post mortem and tuberculosis was found among 1,979 or .01 per cent.[18] The ratio of the occurrence of in-

fection for the two species was 225 to 1.

The explanation for the marked difference in the incidence of tuberculosis in swine compared to that in cattle became apparent many years ago when it was established that more than 80 per cent of the infections in swine were due to the bacillus of avian tuberculosis. Furthermore, it was recognized that a significant correlation existed between the incidence of tuberculosis in swine and the frequency of the disease in poultry in the areas where the infected swine originated.

The fallacy of expecting tuberculosis of swine to disappear with the elimination of the disease in cattle is obvious. Tuberculosis will remain an unnecessary economic burden on swine husbandry until the disease is eliminated from chickens and other barnyard poultry. That the incidence of tuberculosis in swine in the United States is gradually but definitely lessening is indicated in Table 12.3.

Human beings.[19] The literature contains a considerable number of instances in which it was claimed that avian tubercle bacilli were responsible for a tuberculous infection in human beings. Very few of the published reports of such cases contain unequivocal proof necessary to substantiate the contention that avian tubercle bacilli were the agents responsible for the condition described. As a matter of fact, in only a relatively small number of instances has the evidence necessary to prove an avian type of infection in man been presented.[20] The rarity of such cases can only indicate that human beings are extremely resistant to this form of the tubercle bacillus.

[16] Timoney (1939) reported an instance in which tubercle bacilli proven by proper tests of pathogenicity to be avian were obtained from the tuberculous udder of a 7-year-old cow. The report also includes a valuable review of the literature pertaining to infection of cattle by the organisms of avian tuberculosis. Fincher et al. (1954) reported an instance of avian tuberculous infection of the cerebral and spinal meninges and the uterus of a 3 year-old Guernsey cow.

[17] For a detailed account of tuberculosis of swine, the report by Karlson (1961) may be consulted.

[18] [Agriculture Department] Meat Inspection Division, Agricultural Research Service, U.S.D.A. Agricultural Research Service 93-2-6. U.S. Government Printing Office, Washington, D.C. (1962).

[19] Detailed accounts of avian tuberculosis infections in human beings have been published previously by Fontana (1935), Feldman (1938 and 1947), and Rich (1951). Also see Table 12.2.

[20] Several proven cases of infection of human beings with avian type tubercle bacilli have been reported during the past few years. For details see Bradbury and Young (1946), Finlayson (1948), Dragstedt (1949), Karlson and associates (1953, 1955), Furniss et al. (1961), and Cheung and Konst (1965).

TABLE 12.3

INCIDENCE OF TUBERCULOSIS OF SWINE IN FEDERALLY INSPECTED ABATTOIRS
From data compiled by Meat Inspection Division, Agricultural Research Service,
United States Department of Agriculture*

Year Ending June 30	Swine Slaughtered Under Federal Meat Inspection	Swine Affected by Tuberculosis	Per Cent of Swine Affected
1962	67,109,539	1,513,126	2.25
1961	64,209,639	1,587,938	2.47
1960	70,494,427	1,706,136	2.42
1959	63,870,479	1,781,271	2.79
1958	59,202,889	1,645,683	2.78
1957	62,238,519	1,848,466	2.97
1956	66,779,920	2,064,082	3.09
1955	57,055,438	1,898,917	3.33
1954	50,295,636	1,963,276	3.90
1953	57,391,886	2,480,293	4.32

* Data received through the courtesy of Dr. C. H. Pals, Director, Meat Inspection Division, U.S.D.A.

SOURCES OF INFECTION

In most instances the presence of tuberculosis in a farm flock can be explained by the fact that the flock is maintained in an unhygienic environment where infected birds have been kept for many years previously. Of course the introduction of new adult stock from sources where tuberculosis exists is a hazardous procedure and may be the means of transmitting the disease to a previously healthy flock. When new flocks are to be established from sources other than hatcheries, it is exceedingly important that the new stock be from sources where it is definitely known that tuberculosis does not exist. Another possible source of infection is uncooked garbage that may contain the offal of tuberculous fowl and trimmings from tuberculous swine carcasses in which the infectious agent was the avian tubercle bacillus.

Chicks hatched from eggs laid by hens naturally infected with tuberculosis are believed by some to constitute a factor in the transmission of the disease. However, at the present time there is no convincing evidence that tuberculosis is likely to be introduced into a flock in this manner. In other words, baby chicks, regardless of their

maternal source, if reared in an environment free from tubercle bacilli, are unlikely to become tuberculous.[21]

SYMPTOMS IN CHICKENS

In attempting to recognize tuberculosis in the living bird, it should be borne in mind that but few symptoms of the disease are truly pathognomonic. From a practical point of view, probably the most expedient way of diagnosing the disease with certainty is by postmortem examination. The lesions are fairly characteristic and not likely to be confused with other pathologic conditions. However, tuberculous fowl do manifest certain symptoms that, when considered with other factors, constitute presumptive evidence of the disease. It is seldom that any one infected bird will present all the symptoms that have been considered indicative of infection. However, if the disease be at all prevalent in a flock, several or most of the symptoms may be evident in different birds.

Since tuberculosis usually has a protracted course, the disease is more likely to be

* In Norway, where tuberculosis is rare, the disease has been found in migrant wild birds; such birds may account for outbreaks of avian tuberculosis in swine and poultry in that country (Høybråten, 1959).

FIG. 12.3 – Carcasses of three tuberculous chickens from a flock in which the disease was rampant. Note the extreme atrophy of the muscles of the breast.

detected during life by careful and repeated examination than by a single cursory inspection. The incubation period is usually long, and since objective signs are absent in the early stages of the disease, it is usually not possible to diagnose the malady at that time unless resort be had to the tuberculin test.

Ordinarily, if the disease has progressed sufficiently to affect the physical condition of the bird, the animal will be less lively than its mates. If the disease is in an advanced stage the affected fowl fatigues easily and appears depressed or languid. Although the appetite usually remains good, there commonly occurs a progressive and striking loss of weight which often amounts to emaciation. The thinness of the tuberculous bird is especially noticeable in the muscles of the breast (Fig. 12.3). The pectoral muscles are often in a state of complete atrophy, and as a consequence the keel or breast bone becomes strikingly prominent and may be deformed In extreme instances most of the body fat eventually disappears, and the face of the affected bird appears smaller than it would normally

As the disease progresses the feathers assume a dull and ruffled appearance. The comb, wattles, and ear lobes often become anemic and thinner than normal, and the uncovered epidermis has a peculiar dryness. Occasionally, however, the comb and wattles have a bluish discoloration. Icterus, indicative of hepatic changes, may be noted.

Unlike tuberculous infections in many of the mammals, and contrary to the opinion of some writers, the disease in chickens apparently does not induce a febrile state. Even though the disease is most severe, the temperature of the affected bird remains within the normal range. In many instances the bird, when forced to move, reveals a unilateral lameness and walks with a peculiar jerky, hopping gait. This alteration of the gait, which appears to be characteristic, is probably due to tuberculous involvement of the bone marrow of the leg. Infrequently a wing may droop as though paralyzed, owing to a

tuberculous involvement of the bumeral scapulocoracoid articulation. The lesion in this situation may rupture and discharge thin or caseous material. Paralysis due to tuberculous arthritis sometimes occurs, but this is not a frequent symptom of the disease.

If the affected chicken is greatly emaciated, one may detect nodular masses along the intestine by palpation of the abdomen. The great hypertrophy of the liver of many tuberculous birds, however, may make this procedure difficult or impossible. In about 10 per cent of tuberculous chickens, one may recognize the disease by palpation of the involved thymus glands. The crop should be empty if this procedure is to yield satisfactory results.

Most tuberculous chickens have lesions along the intestinal tract, and if these be ulcerative, as they usually are, severe diarrhea that is usually unmanageable results. The enteric disturbance induces an extreme weakness, and the affected bird assumes a sitting position as a result of exhaustion.

The duration of life of the tuberculous chicken is variable. Affected birds may die within a few months or may live for years, depending on the severity or extent of the disease. Death may occur from sheer exhaustion, or the affected bird may die suddenly as a consequence of hemorrhage from rupture of the affected liver or spleen.

But few of the symptoms given are necessarily characteristic of tuberculosis. Convincing proof of a tuberculous infection can be obtained best by necropsy. In fact the importance of necropsy for establishing a diagnosis of tuberculosis in chickens cannot be overemphasized. However, in districts where tuberculous poultry exists, the presence of the disease is suggested by (1) unthriftiness, (2) progressive loss of flesh in spite of good appetite, (3) the chronicity of the symptoms, and (4) the occurrence of the disease in swine not exposed to mammalian tubercle bacilli.

Since the presence of tuberculosis does not preclude the co-existence of other diseases, there are certain conditions that must be differentiated from tuberculosis. These include neoplasia (tumors), tapeworm infection, enterohepatitis, and certain arthritic conditions such as may be associated with fowl cholera, fowl typhoid, paratyphoid, or gout. From the standpoint of the pathologic findings, two facts should be borne in mind in distinguishing tuberculosis from other conditions of chickens. These are (1) the character and distribution of the lesions in the abdomen, associated in a large percentage of cases with lesions in the bone marrow, and (2) the presence within the morbid tissues of numerous acid-fast bacilli. The latter are especially significant since they do not occur in any other spontaneous disease of chickens.

THE TUBERCULIN TEST

At present the method of choice for determining the presence of tuberculosis in the living chicken is the intradermal tuberculin test, which was first applied successfully to chickens by Van Es and Schalk (1914). When it is administered properly and the results are interpreted with understanding, the tuberculin test provides a satisfactory procedure for determining whether or not tuberculosis is present in a given flock. The test is not infallible, but in the hands of one competent to administer it and to interpret the results properly, the procedure offers an extremely valuable aid in the diagnosis and control of avian tuberculosis.

Technique of test. The equipment necessary consists of a sterile tuberculin syringe of the Luer type of 1 cc. capacity and a goodly supply of sterile hypodermic needles one-half inch (1.3 cm.) in length and of 25 to 26 gauge. Absorbent cotton and a few fluid ounces of 70 per cent alcohol should also be available. The tuberculin to be used should be that prepared for intradermic use from avian tubercle ba-

FIG. 12.4.— Positive reaction in left wattle of a tuberculous chicken 48 hours after intracutaneous injection of avian tuberculin.

cilll.[22] The bird should be restrained so that the head is entirely immobile. The site of injection is the wattle. If soiled, the surface of the wattle should be cleaned with alcohol; otherwise, cleaning or attempting to disinfect the skin is unnecessary. The operator grasps the wattle between the thumb and forefinger of one hand, and with the other manipulates the syringe containing the tuberculin. The needle of the syringe then is inserted carefully into the lateral aspect of the dermis, and 0.03 to 0.05 cc. of tuberculin is forced into the tissue. If the procedure has been accomplished properly, a small bleb or a small diffuse blanched area will appear where the tuberculin was deposited. Although fairly satisfactory results may follow if the tuberculin is injected into the subcutaneous tissue, it is a better practice in all instances to place the tuberculin intradermally.

* Tuberculin prepared from mammalian strains of tubercle bacilli may elicit positive reactions in tuberculous chickens, but the results are generally unsatisfactory. More infected birds will be revealed with the avian product, and the reactions to avian tuberculin are usually more pronounced than those elicited with mammalian tuberculin.

The reaction. After 48 hours the chickens are examined and the results recorded. Using for comparison the opposite uninjected wattle, positive reactions are usually easy to recognize, although much experience and a thorough understanding of all factors involved are essential if the results are to be evaluated properly. A positive reaction is indicated by the presence of a soft swelling in the tissues of the injected wattle (Fig. 12.4). Not all reactions are of equal magnitude; some are small, and some result in a pronounced swelling which increases the thickness of the wattle one to five times. The swelling is due largely to edema which occurs in the zone of the connective tissue which lies between the layers of the reflected dermis. To a lesser extent the swelling is due to the increased width of the corium, which is filled with closely packed mononuclear histiocytic cells, a few eosinophilic granulocytes and a variable number of lymphoid cells and lymphocytes (Fig. 12.5). If the reaction is severe the cellular response occurs throughout the corium of the entire wattle. Necrosis of the tissues overlying the site of reaction rarely, if ever, occurs. Hyperemia of the region of the reaction is not apparent, and the swollen wattle is usually grayish or pale yellow. After 48 hours the swelling gradually subsides and usually disappears within 5 days after the tuberculin was injected.

Certain aspects of the test may occasion confusion to some, and in interpreting the results one should keep in mind these factors: Fairly frequently a negative reaction will result in a bird that is definitely tuberculous, and conversely, a positive result is sometimes obtained in chickens in which signs of tuberculosis cannot be demonstrated. In the latter instance, failure to find lesions of tuberculosis does not imply necessarily that tubercle bacilli are not present in the tissues of the chicken. If the disease is in an early stage, lesions are likely to be too small to be noted grossly or too few to be found by the ordinary methods of examination. In a satisfactorily large number of instances a definitely posi-
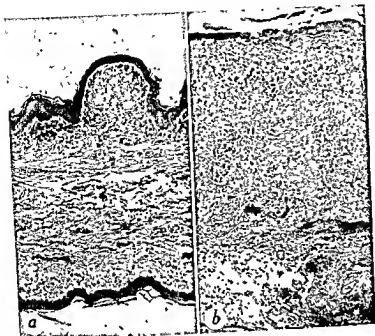
FIG. 12.5 — Cross sections of wattles. (a) Uninjected wattle showing the central connective tissue stroma and the epidermis of the opposite surfaces. (b) Wattle of tuberculous chicken 48 hours after the injection of avian tuberculin, showing characteristic markedly hyperplastic dermal tissue. Both ×56.

tive tuberculin test in chickens indicates that the bird has been exposed to avian tubercle bacilli. If a sufficiently diligent search is made by methods that are proper and adequate, the infective bacteria can usually be demonstrated in positive reactors.

Tuberculin is a bacteria-free substance prepared from the metabolic products of tubercle bacilli and, as used for the diagnosis of tuberculosis in chickens, may be considered entirely harmless to tuberculous as well as to normal birds. Frequently the question is raised whether or not tuberculin injected into nontuberculous fowl may be responsible for a positive reaction on repetition of the test in the same bird. If retests are done after an interval of one month, false positive reactions will not occur. In other words, in chickens the usual diagnostic dose of tuberculin does not sensitize the nontuberculous animal to subsequent injections of the same product.[23]

The tuberculin test has been utilized to a limited extent in diagnosing tuberculosis of turkeys. However, for the most part the results have been less satisfactory than for chickens. Certain difficulties are encountered also in tuberculin testing of pigeons and ducks. Generally speaking, the test is of limited value in diagnosing tuberculosis in these animals.

Rapid agglutination test. A serologic procedure of possible diagnostic usefulness in tuberculosis of chickens was suggested by the report of Moses et al. (1943). The procedure was modified and its practical application demonstrated later by Karlson et al. (1950).

The principal modification introduced was the use of whole blood rather than blood serum. The essentials of the method for conducting the test at the present time are as follows:

(1) Selection of a suitable strain of avian tubercle bacilli for preparation of the antigen. The strain should be one that will produce a uniform and stable suspension. Obviously those strains that auto-agglutinate are not acceptable, and it may be necessary to examine many antigens before one obtained from a suitable culture is found.

(2) Blood for the tests is obtained by pricking the comb with a sharp instrument,

[23] Those interested in the relative value of modifications of the tuberculin test as applied to the wattles of chickens may consult Unruh (1959).
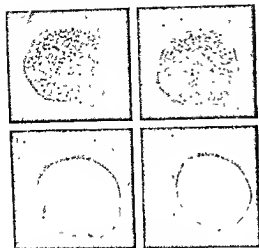
FIG. 12.6 — Results of four tests from four different chickens, two of which were tuberculous and two of which were not.

The two preparations illustrated in the upper row show characteristic agglutination. The blood was obtained from chickens that had reacted positively to tuberculin, and lesions of tuberculosis were found at necropsy. Preparations shown in the lower row failed to agglutinate. The negative reactions agreed with the results of tuberculin tests, and at necropsy lesions were not found. (From Karlson et al., 1950. Reproduction by permission of the American Journal of Veterinary Research.)

such as the point of an 18-gauge hypodermic needle. A drop of the blood is mixed on a warm plate with one drop of the antigen. The plate is kept warm and results are recorded in one minute. The appearance of negative and positive results is shown in Figure 12.6.

Limited observations indicate that the whole-blood agglutination test has a diagnostic reliability in chickens comparable to the tuberculin test. This test should be a relatively rapid and satisfactory procedure for detecting infected flocks. The procedure should be subjected to more extensive trials since it offers certain practical advantages. The animals need to be handled only once, and in addition, samples of blood submitted for agglutination tests for pullorum disease may also be examined for the presence of specific mycobacterial agglutinins.[24]

[24] The reliability of the rapid agglutination test as a diagnostic procedure for the detection of tuberculosis in turkeys should be explored.

PATHOLOGIC ANATOMY

If a proper understanding of the disease problem as it affects the chicken flock is to be obtained, it is essential that a careful necropsy be made of all birds that die. Such an examination, if conducted by one who has expert knowledge of disease, will supply information that can be secured in no other way and will reveal the cause of death in a large percentage of instances. This is especially true in tuberculosis, in which the morphologic signs of the disease are fairly characteristic.

The gross morbid changes associated with tuberculosis of chickens that have died of the disease are usually strikingly evident. If the bird has died suddenly, one frequently finds the abdomen filled with blood. The liver or spleen or both of these organs are greatly enlarged, and the source of the blood can be traced to rupture of one of these organs. Since birds suffering from leukosis may die as a consequence of rupture of the spleen or liver, additional evidence of tuberculosis must be sought in instances in which death was due to a fatal abdominal hemorrhage.

The pathologic changes in avian tuberculosis are those of an infectious granuloma, and although the lesions have a general similarity to those of tuberculosis in mammals, there are certain characteristic distinctions.

It should be kept in mind that the character of the reaction of the tissues to the tubercle bacillus is not determined entirely by the character of the organism, but also by certain indefinite factors which are inherent in the species harboring the infection.

Anatomic distribution of the lesions. Since in the majority of instances tuberculosis of chickens is initiated by way of the digestive tract, it is not surprising that organs other than the lungs should show the greatest incidence of involvement. Lesions of the disease are seen most frequently in the liver, spleen, intestines, and bone marrow. The tuberculous bacillemia, which probably occurs intermittently and perhaps early in most if not all instances
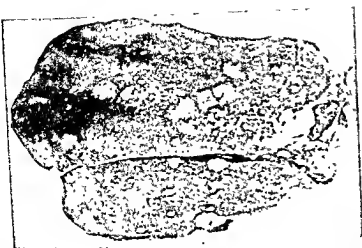
FIG. 12.7 — Tuberculous lesions in the liver of a naturally infected chicken.

FIG. 12.8 — Spleens from chickens naturally infected with tuberculosis. Note the variation in the number and size of the lesions.
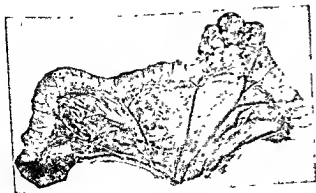
FIG. 12.9 — Large nodulated lesions of tuberculosis in the wall of the small intestine of a chicken.

of tuberculous infection of chickens, provides a favorable circumstance for a widespread or generalized distribution of lesions. None of the tissues, with the possible exception of those of the central nervous system, appear to be immune from possible infection. Some of the organs, such as the heart, ovary, testes, and skin, are affected infrequently and cannot be considered organs of predilection. In one series of 100 necropsies I found the lungs to be affected either grossly or microscopically in 48 per cent.

Gross anatomy of the lesions. Grossly, avian tuberculosis is characterized by the occurrence of irregular grayish-yellow or grayish-white nodules of varying sizes in the organs of predilection such as the liver, spleen, intestine, and bone marrow (Figs. 12.7, 12.8, and 12.9). Involvement of the liver and spleen results in hypertrophy which is often of marked proportions. The tuberculous nodule, as observed grossly, varies in size from a structure that is just discernible to a huge mass that may measure several centimeters in diameter.[25] Nodules of large size frequently have an irregular knobby contour, with smaller granulations or nodules often present over the surface. Lesions near the surface in such organs as the liver and spleen are enucleated easily from the adjacent tissues. The nodules are firm but are incised easily since mineral salts are not present. On cross section there may be observed a fibrous nodule containing a variable number of small yellowish foci or a single soft, yellowish central region which is frequently caseous. The latter is surrounded by a fibrous capsule, the continuity of which often is interrupted by small circumscribed necrotic foci. The fibrous capsule varies in thickness and consistency depending upon the size and duration of the lesions. It is barely discernible or apparently absent in the smaller lesions and measures from 0.1 to 0.2 cm. in thickness in the larger nodules.

The number of lesions present is also variable, ranging from a few to innumerable. Large numbers of small lesions are particularly frequent in the liver and in the mesentery. It is fairly common to observe a few nodular lesions in organs such as the liver and spleen associated with an enormous number of lesions of minute to moderate size. The variation in size of such lesions is a consequence of successive episodes of reinfection from previously established lesions, usually of the same organ. Involvement of the lung is usually less severe than that of the liver or spleen (Fig 12.10).

The continuous progressiveness of tuberculosis of chickens, once the disease is established, and the marked tendency of the disease to disseminate to several organs of the body indicate, as mentioned previously, that tuberculous bacillemia is a common manifestation of the disease. That the blood stream of tuberculous fowl does contain virulent tubercle bacilli at times has been demonstrated repeatedly. This tendency of the bacilli to invade, and circulate with the blood stream provides the explanation for the frequent involvement of the bone marrow of tuberculous fowl (Figs. 12.11 and 12.12). Infection of the bone marrow probably occurs very early in the course of the disease and is characterized by hypertrophy of the myeloid tissues, by disappearance of most of the bony spicules, and finally by the formation in the marrow of tuberculous nodules. The latter may be numerous and strikingly evident to the unaided eye, or the lesions may be few and of such size as to require the use of the microscope for their demonstration.

Blood. Reliable data on the effects of a natural tuberculous infection on the circulating blood of chickens are somewhat meager. Some workers have reported anemia associated with a reduction in the total number of erythrocytes. Other observations have indicated that there occurs a marked increase in the number of large lymphocytes and a decrease in the small

[25] Detailed descriptions of the gross and microscopic lesions of tuberculosis in the different organs of chickens will be found in Feldman's (1938) monograph on avian tuberculosis infections.

FIG. 12.10 — Massive tuberculous involvement of one lung of a naturally infected chicken.

FIG. 12.11 — Several femurs and one tibio from chickens naturally infected with tuberculosis showing lesions in the myeloid tissue and some well-marked osteoplastic changes due to the infection.
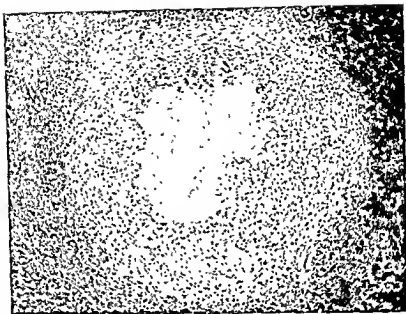
FIG. 12.12 — Small tuberculous nodule in the bone marrow of a naturally infected chicken. The central necrotic region is surrounded by a zone of dense connective tissue. ×100.
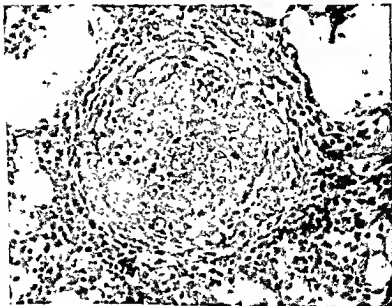
FIG. 12.13 — Young epithelioid tubercle in the lung of a chicken. ×440.

lymphocytes. The work of Olson and Feldman (1936) on a relatively small number of naturally infected chickens indicated that the erythrocyte and thrombocyte counts and the values for hemoglobin were within the limits of normal. Although in our material the disease was presumably of long duration, anemia was not observed. Leukocytosis was the most striking and consistent finding, the number of monocytes and heterophils being increased. The degree of leukocytosis was for the most part in direct ratio to the extent and severity of the disease.

Histopathology of the tubercle. The anatomic unit of tuberculosis as the disease occurs spontaneously is conveniently designated a tubercle. The term "tubercle" as it refers to tuberculosis of fowl designates a structure which varies in character depending on its age and size. In its simplest form, which may be observed experimentally in 10 to 14 days after infection, there occurs a closely packed, microscopic collection of rather pale-staining cells with vesiculated nuclei. These cells, which have been designated as epithelioid cells, contain tubercle bacilli and are derived from fixed tissue elements known as histiocytes (Fig. 12.13). The latter cells have a marked attraction for tubercle bacilli, which they phagocytose early in the reactive process.

The cellular mass, or primary tubercle, gradually expands as a consequence of the proliferative activity of histiocytes at the periphery, and within three to four weeks after the tubercle first becomes demonstrable, signs of retrogression can be detected in the epithelioid cells of the central zone. This retrogression is due in part to the avascularity of the structure and in part to the toxic substances of the tubercle bacilli. As the cellular mass becomes larger, the epithelioid cells have a tendency to fuse and form syncytia. The outlines of individual cells become less distinct or disappear. Vacuoles appear, and the staining reaction is more acidophilic. This is followed within a week or so by a necrobiotic change resembling coagulation necrosis. The nuclei of the epithelioid cells become pyknotic and may disappear, while the cellular mass, excepting the peripheral portion, becomes fused and stains deeply with eosin. The tubercle bacilli have multiplied and appear singly or in clumps throughout the necrotic tissue. This completes the first phase in the evolution of the tubercle.

The second phase of the development of the tubercle is concerned with the formation of giant cells. While the epithelioid cells in the central zone undergo necrobiotic changes, there persists an outer zone of epithelioid syncytia which appears as a mantle around the entire periphery. From these, giant cells are developed. The giant cells thus formed may contain one or several nuclei. Infrequently, forms similar to Langhans' giant cells of mammalian tuberculosis may occur. The nuclei of the giant cells are situated distally to the central zone of necrosis, and the cells are arranged rather frequently in palisade formation. Large vacuoles often occur in the cytoplasm of the giant cells, and the nuclei stain intensely with the basic dyes. Immediately peripheral to the zone of giant cells there occurs a more or less diffuse collection of epithelioid cells and their progenitors, histiocytes (Fig. 12.14). Fibrocytes and minute blood vascular channels also occur near the outer portion of the peripheral area. Although tubercle bacilli are more numerous in the central or necrotic zone of the tubercle, they also occur in large numbers in the epithelioid zone, adjacent and distal to the giant cells.

The third and final phase in the formation of a tubercle is the development of a zone of encapsulation consisting of fibrous connective tissue, histiocytes, some lymphocytes, and an occasional eosinophilic granulocyte. In limiting the progress of the disease the encapsulating structure is usually inadequate owing to the continuous development of new tubercles in the epithelioid zone immediately peripheral to the giant cells. As a consequence of these new or so called daughter tubercles, a tubercle as recognized grossly consists of the original or parent tubercle and several

FIG. 12.14 — Developing tubercle in the lung of a chicken showing activity
of the third or tuberculogenic zone. ×100.
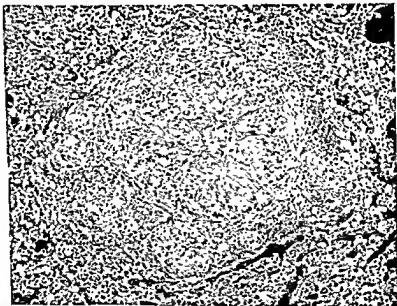
FIG. 12.15 — Conglomerate tubercle in the lung of a chicken. Numerous
secondary tubercles are present in the outer or peripheral part of the lesion.
×120.

smaller or adjacent ones, which considered together form a conglomerate tubercle (Fig. 12.15).

In summary, it is convenient to consider that the adult tubercle as it occurs in chickens consists of four parts or zones. The first is the necrotic or central zone, and the second, the surrounding zone of giant cells. The third zone is that immediately peripheral to the giant cells and is composed of epithelioid cells and histiocytes. The fourth zone, which is not always apparent, is made up of histiocytes, small blood channels, and fibrous connective tissue elements.

The nature of the degenerative process which occurs in the central zone of the tubercle is somewhat unusual in that the integrity of the cells is maintained for a considerable period before disintegration becomes apparent. Caseation necrosis eventually occurs and may affect all or a part of the central zone. Caseation probably is engendered by the influx of leukocytes, and there results a more or less structureless mass composed of tissue debris and nuclear fragments among which tubercle bacilli are numerous.

By appropriate stains, lipid substances in variable amounts can be demonstrated in the lesions. The fat, which occurs in the form of small to moderately large globules, is most abundant in the more adult tubercles and of minimal amount in the prenecrotic epithelioid lesions.

In the first or epithelioid phase of the development of the tubercle, one can demonstrate by appropriate staining methods the presence of delicate reticulum fibrils which intertwine promiscuously among the epithelioid cells. When degeneration and necrosis occur, the reticulum fibrils no longer can be seen.

Calcification of the tubercle occurs rarely if ever, the failure of mineral salts to accumulate being one of the unique characteristics of the tuberculous lesion as it occurs in fowl. Amyloidlike degeneration of portions of the surrounding parenchymal elements sometimes is observed in the liver, spleen, and kidney.

The number of tubercle bacilli present in the lesions is much significance in the pathogenesis of avian tuberculosis. The propensity of the organism for growth and multiplication is hindered little if any within the tissues of chickens, and the result is prodigious numbers of bacilli in every lesion (Fig. 12.16). In this regard tuberculosis of chickens resembles two other mycobacterial diseases—leprosy and paratuberculosis. The organisms are exceedingly numerous in smears from the morbid tissues, and cultures of tuberculous tissue from chickens usually yield innumerable colonies of tubercle bacilli. The presence of tubercle bacilli in such large numbers within the tissues of a tuberculous fowl constitutes an important factor in the transmission of the disease to healthy animals and makes imperative the proper disposal of the carcasses of birds affected with tuberculosis.

Generally speaking, the morbid anatomy of tuberculosis of chickens is that of a serious, destructive, aggressive, granulomatous disease that seldom if ever heals spontaneously and, in the great majority of instances, will result directly or indirectly in the death of the affected fowl.

DISSEMINATION AND TRANSMISSION

Although several factors may contribute to the transmission and dissemination of avian tuberculosis to uninfected hosts, an infected environment is the element of first importance in the perpetuation of the disease. Should a high percentage of infection occur as a consequence of an infected environment, several related factors are of significance. These include: (1) the age of the chicken, adult chickens being more resistant than younger ones; (2) the concentration of the infective material, premises occupied by many tuberculous fowl over a period of years being a more potent source of infection than premises that have been contaminated recently by relatively few tuberculous fowl; (3) repeated episodes of exposure over a considerable period; and (4) the complex and little-understood question of individual
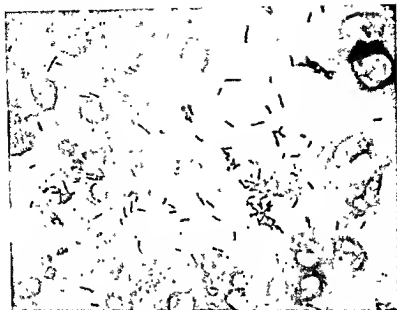
FIG. 12.16 – Numerous tubercle bacilli in a smear preparation from a small lesion of the lung of a naturally infected chicken (stained by the method of Ziehl-Neelsen). ×1,600.

susceptibility or resistance.

Of primary importance in the establishment of an infected environment are certain distinctive factors characteristic of the pathology of avian tuberculosis in the natural host. As mentioned before, the tremendous number of tubercle bacilli exuded from the frequently occurring ulcerated tuberculous lesions of the intestine establish the infected bowel as a constant source of virulent bacteria. These mix with the intestinal contents and eventually leave the body with the feces. Although other potential sources of infection exist, there is none that equals infective fecal material in the dissemination of avian tuberculosis from affected to nontuberculous animals. Related to enteric ulcerations as sources of tubercle bacilli that may occur in the fecal discharges are lesions of the liver and of the mucosa of the gallbladder. From such lesions the organism fairly commonly finds its way into the intestine through the common duct.

Egress of the bacteria from the respiratory tract is also a potential source of infection, especially if lesions occur in the tracheal mucosa in addition to the lungs. The ingestion of its infective exudates by a bird that has tuberculosis of the respiratory tract also provides a potent means of auto-infection, with lesions in the other organs, especially the intestines.

Vectors. The possibility that living foreign-host carriers may transport avian tubercle bacilli from infected to noninfected premises constitutes an interesting phase of the epidemiologic aspect of avian tuberculosis. Many have studied the problem, the report of Schalk et al. (1935) being especially noteworthy. Although a résumé of the facts indicates quite definitely that vectors have a role in the dissemination of avian tubercle bacilli from infected to healthy flocks, it is hardly likely that vectors are responsible for any considerable amount of the tuberculous infection that exists in the average farm flock. They are perhaps more important as possible sources of new foci of infection in premises that were previously free of the disease. The infective environment,

comprising as it does the bacilli-laden soil, litter, and filth, is the factor of greatest importance in the transmission of the disease to noninfected animals. The longer the premises have been occupied by infected birds and the more concentrated the poultry population, the more prevalent the infection is likely to be.

Role of eggs. The possibility that avian tuberculosis might be transmitted through the eggs from tuberculous hens has been a pertinent question since Sibley (1890) observed the occurrence of tuberculosis in chickens that had been hatched from eggs laid by hens affected with the disease. In attempting to explain the origin and continuation of the disease, Sibley stated that "the disease appears to be a clear case of heredity."

Generally speaking, the problem concerning the possible transmission of avian tuberculosis through infected eggs has been approached in two ways: (1) by inoculating eggs artificially with tubercle bacilli and noting whether or not tuberculosis eventually develops in the birds hatched from the infected eggs and (2) by observing whether or not tuberculosis develops in chickens hatched from eggs obtained from naturally infected hens.[26] It has been demonstrated many times that some of the eggs artificially inoculated with avian tubercle bacilli will hatch and that there is a good possibility that the chicks hatched from such eggs will be infected with tubercle bacilli. Furthermore, such infected chicks usually will die within a short time of extensive tuberculosis. Therefore, from a practical point of view, such observations are of doubtful importance to the fundamental question: Are eggs from naturally infected chickens likely to produce chicks that are destined to be tuberculous? Although the possibility that this might occur is admitted, there is at the present time no convincing experimental evidence to justify the conclusion

that chicks hatched from eggs laid by tuberculous hens will be infected with tubercle bacilli as a consequence of the infectious agent having been implanted in the developing embryo.

The most convincing evidence that infection from a tuberculous maternal parent is not likely to occur is that furnished by the investigations of Schalk et al. (1935) and Fitch and Lubbehusen (1928), who reared many hundreds of chicks hatched from eggs of naturally infected hens without tuberculosis having been observed in a single instance.

Other sources. Other potent sources of dissemination of avian tubercle bacilli are the carcasses of tuberculous fowl that die of the disease and the offal from chickens that, although tuberculous, are dressed for food. It is obvious that any tissue likely to contain living avian tubercle bacilli should be disposed of in such a manner as to preclude its being eaten by chickens or swine. Infected tissues preferably should be burned, or if the food value of such flesh be of sufficient importance, it should be cooked thoroughly before being used as food for animals.

It is also conceivable that cannibalism might play a part in the transmission of tuberculosis from one chicken to another. Since bacillemia is of frequent occurrence in the natural course of tuberculosis of chickens it is reasonable to believe that the fierce and bloody assault on an infected bird by one addicted to cannibalism would provide a possibility that the aggressor would ingest tubercle bacilli with the blood of the infected victim. Whether or not such exposure would be sufficient to produce tuberculosis is problematic.

Avian tubercle bacilli may be transmitted from one situation to another by persons whose shoes have become soiled with fecal matter and other filth of the poultry yard. The equipment used in the care and maintenance of infected poultry flocks, such as crates and feed sacks, also might be responsible for the transfer of the infective bacteria from diseased to healthy flocks.

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[26] Fitch et al. (1924), as a result of a comprehensive study, concluded that viable tubercle bacilli are present in less than 1 per cent of eggs from tuberculous chickens

TUBERCULOSIS IN TURKEYS[27]

Turkeys are not uncommonly affected with tuberculosis, and the disease in most instances is contracted from infected chickens. The disease is chronic in character. Affected birds may occasionally be lame or emaciated. However, such signs are not pathognomonic. Definitive diagnosis is best established as a result of necropsy procedures.

Important information regarding tuberculosis in turkeys has been contributed by Hinshaw et al. (1932), who examined at necropsy a total of 88 birds; tuberculosis was found in 45, or 51.14 per cent. The incidence of the disease in relation to different age groups is of interest. The disease was found in only one of 11 birds less than one year of age. Of 24 between one and two years of age, 14 were tuberculous, whereas of 43 more than two years of age, 28, or 65.12 per cent, were tuberculous. In two instances the age of the birds was not known. These workers recorded the frequency of involvement of the different organs as follows: liver, 95.6 per cent; spleen, 67.3 per cent; intestine, 45.6 per cent; lungs, 32.6 per cent; ovaries, 32.2 per cent; thymus, 29 per cent; testes, 25 per cent; mesentery, 21.7 per cent; pancreas, 12.9 per cent; muscle, 11.7 per cent;[28] bones, 7.4 per cent; skin, 6.2 per cent;[29] gizzard, 5.7 per cent; esophagus, 4.7 per cent; pericardium, 4.5 per cent; proventriculus, 3.7 per cent; kidneys, 3.5 per cent; oviduct, 3 per cent; and myocardium, 2.2 per cent.

Microscopically, lesions of tuberculosis in the turkey vary considerably in their appearance. In some, tubercles essentially like those seen in tuberculosis of chickens are present. In other instances, the lesions are of a diffuse character with extensive destruction of the surrounding parenchymal tissues. Cytoplasmic masses, or large giant cells, may be numerous, and large numbers of eosinophilic granulocytes are commonly present. Some of the lesions become circumscribed by a broad, dense zone of fibrous connective tissue. At the periphery of such encapsulations, extensive infiltrations of lymphocytes are usually present.

As mentioned previously, the diagnosis of tuberculosis in turkeys is best accomplished by recognizing the lesions at necropsy. However, proof of the true nature of the infection is dependent upon subsequent laboratory studies designed to reveal the acid-fast characteristic of the etiologic agent and to establish the microorganism definitely as Mycobacterium avium. Conditions that may simulate tuberculosis and which must be excluded are mycosis, enterohepatitis, and certain forms of neoplasia.

From available information it appears that the usefulness of the tuberculin test in the diagnosis of tuberculosis in turkeys has not been adequately established. Hinshaw et al. (1932) using avian tuberculin, injected the snood, the mucosa of the anus, the wattle, the skin of the edge of the wing web, and the skin at the center of the wing web. The results of the tests indicated that (1) the reactions in the wattle agreed with the findings at necropsy in only 11.1 per cent of the birds, and (2) there was agreement between the tuberculin reaction in the wing web and the findings at necropsy in 75.68 per cent of the animals. Durant (1936) also found the skin of the wing web to be the site of choice for tuberculin testing of turkeys. From the meager information available, one must conclude that the intradermic tuberculin test has been less reliable for detecting tuberculosis in turkeys than in chickens. It would seem desirable to investigate the reliability of the rapid agglutination test as

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[27] Information pertaining to tuberculosis in turkeys has been contributed by Eber (1924, 1925), Klimmer (1930), Proscholdt (1932), Sustmann (1917), Hinshaw et al. (1932), and Hinshaw, Chapter 41 of this book.

[28] The lesions designated as involving muscle were, according to Hinshaw et al. (1932) actually in the fascia.

[29] Instances of tuberculosis of the skin and of the subcutaneous tissues of turkeys have been described by Scrivner and Elder (1931), Chrétien et al (1923), and Dietrich (1927).

a means of detecting tuberculosis in live turkeys.[30]

Recommendations for the control of tuberculosis in turkeys include the following: (1) Rear and maintain birds so as to prevent contact with tuberculous chickens; premises and housing previously used by tuberculous chickens are to be avoided. (2) When tuberculosis is discovered in a flock of turkeys, the entire flock should be disposed of. (3) If a flock is to be re-established, the new stock should be limited to day-old poults.

CONTROL OF AVIAN TUBERCULOSIS

The eradication of avian tuberculosis or even its satisfactory control is not a simple matter. However, the widespread distribution of the disease, its high incidence in the more seriously infected areas, and the increasing importance of the poultry and the swine industries make it imperative that adequate measures be devised for its control and ultimate eradication.

As in most other infectious diseases, vaccination for the prevention of avian tuberculosis has been considered and tried. The products used include the so-called Friedmann vaccine,[31] an avian strain of BCG, and heat-killed avian tubercle bacilli. The result obtained from any of these products would not justify the claim that avian tuberculosis as the disease occurs naturally can be controlled successfully by vaccination. A measure of resistance can be conferred by the use of homologous strains, but much additional work will be necessary before vaccination can be accepted as worthy of serious consideration in the prevention of the disease.[32]

The tuberculin test if used judiciously is of considerable practical value in reducing the losses from tuberculosis. The subsequent removal from the flock of

chickens that react eliminates many foci of infection. The test enables one to detect many infected fowl before the disease reaches a severe or chronic state, and if repeated tests are made, potential dissemination of the infective bacteria to the surrounding environment may be reduced appreciably. However, this method if depended on alone for combating avian tuberculosis has many shortcomings, the most important being that if the residual flock is permitted to occupy the same infective premises a continuing source of infection remains. This provides opportunity for new infections to occur for an indefinite period since in the soil avian tubercle bacilli may remain viable and virulent for years. For this reason an environment once infected remains a potential source of infection indefinitely. Furthermore, neither the tuberculin test nor any other means can be depended on with absolute certainty to detect every living tuberculous fowl, and as long as one infected bird remains in a flock, dissemination of the disease to healthy fowl is possible. Consequently, means other than the tuberculin test must be resorted to if a more satisfactory control of avian tuberculosis is to be expected.

For the past several years it has been frequently stated that avian tuberculosis can be controlled if all birds in the flock are disposed of after the first laying season. This practice has much to commend it, especially since it is economically sound from the point of view of egg production. Older birds usually produce fewer eggs than the younger ones, and furthermore, the mortality from nonbacterial diseases such as neoplasia is greater among the older hens than among pullets. Another factor in favor of the disposal of the older stock is that if tuberculosis is present it is usually more severe in the older birds, which are as a consequence more likely to become depots of dissemination.

Desirable as it may be to dispose of the older birds, to maintain that this practice alone will rid the flock of tuberculosis

[30] Referred to previously (page 389) as a diagnostic test for tuberculosis in chickens

[31] Prepared from a so-called turtle strain of acidfast bacilli (*Mycobacterium chelonei*).

[32] The question of vaccination against tuberculosis of chickens is reviewed in Feldman's (1938) monograph on avian tuberculosis infections.

is at best an optimistic wish. Contrary to the belief of some, acute generalized tuberculosis occasionally occurs in pullets. The lesions in such a bird contain enough tubercle bacilli to infect a dozen flocks, and if the carcass be eaten by its mates, the likelihood that several additional birds will become infected is evident. To reiterate, the threat of avian tuberculosis in potentially serious proportions remains just so long as a single infected bird is a member of the flock.

Since it has been adequately established that the continuation of tuberculosis in a flock is dependent on an infected environment, it would seem reasonable to believe that this fundamental fact should be utilized in any program devised for the control and elimination of the infection. Basically the question is one of hygiene. That every case of tuberculosis comes from another case is aphorismic. The disease is due to a well-known and definitely established cause, the tubercle bacillus, and this fact must not be lost sight of or ignored when measures to eliminate the infection are considered. To permit young birds to range at will over infected premises or to occupy quarters that were used previously to maintain tuberculous birds is to insure the continuation of the disease indefinitely.

Procedures for establishing and maintaining tuberculosis-free flocks should embrace the following: (1) Abandon the old equipment and establish other facilities on new soil that is known not to be contaminated with avian tubercle bacilli. Ordinarily it is impractical to render an infected environment satisfactorily safe by disinfection. (2) Provide proper fencing or other measures to prevent the unrestricted movement of the chickens, thus preventing exposure from previously infected premises. (3) Eliminate as soon as possible the old flock, burning the carcasses of birds that show lesions of tuberculosis. (4) Establish a new flock in the new environment from tuberculosis-free stock. (5) Eliminate from the swine herd all reactors to avian and to mammalian tuberculin. New breeding stock should likewise be tuberculosis-free. If the chickens in such a flock are prevented from having access to an infected environment and are protected against accidental exposure to tubercle bacilli, it is reasonable to believe that they will remain free from tuberculosis.

The measures just mentioned for the elimination of avian tuberculosis are not complicated and should be applicable to most American farms. The additional profits that will accrue from a tuberculosis-free flock maintained in a hygienic environment will in time compensate adequately for the initial expense and work necessary to establish the new flock and new facilities. Furthermore, the general health of the birds will be better, and diseases other than tuberculosis will be controlled more satisfactorily. The benefits will also be reflected in a decrease in tuberculosis in swine. The importance of avian tuberculosis in the infection of swine is such that if chickens were maintained entirely separate and apart from swine, the incidence of tuberculosis of swine due to $M.$ $avium$ would be reduced to a minimum.

Some may be tempted to try to control or eliminate avian tuberculosis from infected birds and mammals by the use of antituberculosis drugs which have been effective in the treatment of tuberculosis in man. Such practice cannot be recommended. The organism of avian tuberculosis is characteristically much more resistant to presently known chemical agents in $vivo$ than is the human tubercle bacillus. Furthermore, the tuberculosis infection in chickens is often a formidable, destructive process, with hematogenous dissemination frequently, if not always, present. The procurement of a chemical agent sufficiently potent to eliminate the infection without serious untoward results on the affected animal seems unlikely and visionary at the present time. One may again emphasize: tuberculosis of poultry is best combated by elimination from the premises of all natural hosts infected with $Mycobacterium$ $avium,$ and by strict adherence to basic principles of sanitation and hygiene.

REFERENCES

Anderson, R. J.: 1942. The chemistry of the lipids of the tubercle bacillus. Yale Jour. Biol. and Med. 15:311.

———, and Creighton, M. M.: 1939. The chemistry of the lipids of tubercle bacilli. LVII. The mycolic acids of the avian tubercle bacillus wax. Jour. Biol. Chem. 129:57.

———, Creighton, M. M., and Peck, R. L.: 1940. Chemistry of lipids of tubercle bacilli. LX. Concerning the firmly bound lipids of the avian tubercle bacillus. Jour. Biol. Chem. 133.675.

———, and Roberts, E. G.: 1930a. The chemistry of the lipoids of tubercle bacilli. Jour. Biol Chem. 85:509.

———, and Roberts, E. G.: 1930b. The chemistry of the lipoids of tubercle bacilli. XI. The separation of lipoid fractions from avian tubercle bacilli Jour. Biol Chem. 85:519.

———, and Roberts, E. G.: 1930b. The chemistry of the tubercle bacilli Jour. Biol. Chem. 85:519. phosphatide fraction of the avian tubercle bacilli. Jour. Biol. Chem. 85:519.

Bradbury, F. C. S., and Young, J. A.: 1946. Human pulmonary tuberculosis due to avian tubercle bacilli — Report of a case. The Lancet 1:89.

Chargaff, E., and Moore, D. H.: 1944. On bacterial glycogen: the isolation from avian tubercle bacilli of a polyglucosan of very high particle weight. Jour. Biol Chem. 155:493.

Cheung, O. T., and Konst, H.: 1963. Pulmonary tuberculosis apparently caused by the avian tubercle bacillus. Canad. Med Assn. Jour. 89-116

Chrétien, A., Germain, and Raymond: 1923. Anatomie pathologique de la tuberculose aviaire. Rev. de la tuberc. 4:25

Christiansen, M., Ottosen, H., E., and Plum, N.: 1946. A peculiar infection with acid fast bacteria in wood-pigeons (Columba palumbus L.). Skand., Veterinar-Tidskr. 36:352.

Cornil, V., and Mégnin, P.: 1884. Tuberculose et diphthérie des gallinacés, Compt. rend. Soc. de biol. 36.617.

Dietrich, A.: 1927. Ein Fall von Hauttuberkulose bei einer Pute. Berliner tierärztl. Wochenschr. 43:294.

Dragstedt, L.: 1949. Avian tuberculosis in man. The Lancet 257:103.

Durant, A. J.: 1936. Tuberculosis of poultry. Univ. Mo. Agr. Exper. Sta., Bul. No. 364, 22 pp.

Eber, A.: 1924. Die Tuberkulose des Hausgeflügels. Zeitschr. f. Infekt-Krankh. 25:145.

———: 1925. Ibid. 27:1.

Feldman, W. H.: 1938. Avian Tuberculosis Infections. The Williams and Wilkins Company, Baltimore, 483 pp.

———: 1947. Animal tuberculosis and its relationship to the disease in man. Ann, N.Y. Acad. Sc. 48 (Art. 6) :469.

———: 1960. Avian tubercle bacilli and other mycobacteria — Their significance in the eradication of bovine tuberculosis. Am. Rev. of Respir. Dis. 81(5).666.

Fincher, M. G., Evans, W. M., and Saunders, L. Z.: 1954. Avian tuberculosis in a dairy cow. Cornell Vet. 44:240.

Finlayson, M. K.: 1948. Case of human tuberculosis due to avian tubercle bacilli. New Zealand Med. Jour. 47:562.

Fitch, C. P., and Lubbehusen, R. E.: 1928. Completed experiments to determine whether avian tuberculosis can be transmitted through the eggs of tuberculous fowls. Jour. Am. Vet. Med. Assn. 72:636.

———, Lubbehusen, R. E., and Dikmans, R. N.: 1924. Report of experimental work to determine whether avian tuberculosis is transmitted through the eggs of tuberculous fowls. Jour. Am. Vet. Med. Assn. 66.43.

Fontana, A.: 1935. La Tuberculosi Aviaria. Milan. Casa Editrice Dottor Francesco Valiardi. 179 pp.

Furniss, A. L., Collins, C. H., and Marks, J.: 1961. A case of infection with avian type tubercle bacilli. Great Britain Ministry of Health, Monthly Bulletin, Public Health Laboratory 20:126.

Hall, R. E., and Winkel, F.: 1957. Avian tuberculosis in mink. A case report. Jour. Am. Vet. Med. Assn. 131:49.

Hastings, E. G., and Halpin, J. G.: 1913. Avian tuberculosis. Univ. Wis. Agr. Exper. Sta., Res. Bul. 28.

Hays, C. H.: 1929. Avian tuberculosis in Nebraska. Jour. Am. Vet. Med. Assn. 75:549.

Hinshaw, W. R.: 1933a. Tuberculosis of Avian Origin in Muscovy Ducks. Jour. Am. Vet. Med. Assn. 82.111.

———: 1933b. Tuberculosis of Human Origin in an Amazon Parrot. Am. Rev. of Tuberculosis 28.273.

———, Niemann, K. W., and Busic, W. H.: 1932. Studies of tuberculosis of turkeys. Jour. Am. Vet. Med. Assn. 80-765

Høyberket, P.: 1959 Tuberkulosetilfeller hos fugler. Nord. Veterinaermed. 11:780.

Jollès, P., Bigler, F., Gendre, T., and Lederer, E.: 1961. Sur la structure chimique du "Mycoside C" peptido-glycolipide de Mycobacterium avium. Bul. Soc. Chim. Biol. 43:177.

Karlson, A G.: 1961. Tuberculosis in swine. In Diseases of Swine (Chap. 27), edited by H. W. Dunne, Iowa State University Press, Ames, Iowa

———, Andersen, H. A., and Needham, G. M.: 1955. Isolation of avian tubercle bacilli in human silicosis. Diseases of the Chest.

———, and Feldman, W. H.: 1953. Mycobacteria of human origin resembling Mycobacterium avium. Proc. XV Internat. Vet. Cong. Part 1, 1:159.

Karlson, A. G., Zinober, M. R., and Feldman, W. H: 1950. A whole blood, rapid agglutination test for avian tuberculosis—A preliminary report. Am. Jour. Vet. Res. 11:137.

Klimmer, M.: 1930 Die Übertragung der Geflügeltuberkulose auf Menschen und das Vorkommen von Tuberkelbacillen in Hühnereiern. Berliner tierärztl. Wochenschr. 46.702.

Koch, R.: 1882. Die Aetiologie der Tuberkulose. Berliner klin. Wochenschr. 19.221.

———: 1890. Ueber bakteriologische Forschung. Wien. med. Bl. 13.531.

———: 1902 Address before the Second General Meeting. Tr. Brit. Cong. Tuberc. 1:25.

Konno, K· 1956. New chemical method to differentiate human-type tubercle bacilli from other mycobacteria Science 124.985.

Long, E R: 1958. The chemistry and chemotherapy of tuberculosis. Third Ed. Williams & Wilkins Company, Baltimore 450 pp.

McDiarmid, A. 1948 The occurrence of tuberculosis in the wild wood-pigeon. Jour. Comp. Path and Therap. 58·128.

Mac Lennan, A P· 1962 The monosaccharide unit in specific glycolipids of Mycobacterium avium Biochem. Jour 82.394.

Maffucci, A 1890 Beitrag zur Aetiologie der Tuberkulose (Hühnertuberkulose). Zentralbl. f. allg Path. u. path. Anat. 1 409

Menzel, A. E O., and Heidelberger, M 1938 Cell protein fractions of bovine and avian tubercle bacillus strains and of the timothy grass bacillus. Jour. Biol. Chem. 124·501.

Mitchell, C A., and Duthie, R C 1950 Tuberculosis of the common crow. Canad. Jour. Comp. Med and Vet. Sci 14 109

Miquel, Anne-Marie, Ginsberg, Hélène, and Asselineau, Jean: 1963. Composition Des Cires C et D De Mycobacterium Avium Bul. Soc Chim. Biol. 45:715.

Moses, H. E., Feldman, W H, and Mann, F C: 1943. Mycobacterial rapid agglutination antigens and their diagnostic value in tuberculosis of fowl. Am Jour. Vet. Res. 4.390.

Noll H· 1957. The chemistry of some native constituents of the purified wax of Mycobacterium tuberculosis. Jour. Biol. Chem 224:149.

Olson, G., Jr, and Feldman, W H 1936. The cellular elements and hemoglobin in the blood of chickens with spontaneous tuberculosis. Jour. Am. Vet. Med. Assn. 89.26.

Proscholdt, quoted by Zeller, H, 1932. Die Tuberkulose des Geflügels. Ergebn. d. all. Path. u. path. Anat. 26 824

Radcliffe, H. L.: 1948 Tuberculosis in captive birds. Decrease of its incidence following a change in diets. Am Rev. Tuberc. 54·389.

Reeves, R. E, and Anderson, R J: 1937. The chemistry of the lipides of tubercle bacilli. XLVII The composition of the avian tubercle bacillus wax. Jour. Am. Chem. Soc. 59 858.

Renfrew, A. G: 1929 A proximate analysis of a defatted residue of avian tubercle bacilli. Jour. Biol. Chem 83·569

Rich, A. R.: 1951 The Pathogenesis of Tuberculosis. 2nd ed. Charles G Thomas, Springfield, Illinois, 1028 pp

Ravolta, G.· Quoted by Maffucci, A.

Rotach, F. 1947. La classification sérologique des bacilles de la tuberculose aviare. Schweiz. Zeitschr. f Path. u. Bakt. 10.335

Runyon, E. H., Selin, M. J., and Harris, H. W.: 1959. Distinguishing mycobacteria by the niacin test—A modified procedure. Am. Rev. Tuberc. and Pul. Dis. 79 663.

Sabin, F. R · 1932 Cellular reactions to fractions isolated from tubercle bacilli. Physiol. Rev. 12·141.

Schalk, A. F., Roderick, L. M., Foust, H. L., and Harshfield, G. S.: 1935. Avian tuberculosis: collected studies. N. Dak. Agr. Exper. Sta., Tech. Bul. 279.

Scrivner, L. H, and Elder, C: 1931. Cutaneous and subcutaneous tuberculosis in turkeys. Jour. Am. Vet. Med. Assn. 79:244.

Sibley, W. K.: 1890. The tuberculous in birds. Jour. Comp. Med. and Vet. Arch. 11:317.

Smith, D. W., Randall, H. M., Mac Lennan, A. P., and Lederer, E.: 1960. Mycosides: a new class of type-specific glycolipids of Mycobacteria. Nature 186 887.

Sussmann, R: 1917. Seuchenhaftes Auftreten der Tuberkulose bei Truthühnern. Münchener tierärztl. Wochenschr. 68.683.

Timoney, J. F· 1959. Avian tuberculosis in a cow. Vet. Record 51:191.

Unruh, W: 1959. Untersuchungen über eine Verbesserung der Technik der Tuberkulinkehllappenprobe beim Huhn (Inaugural-Dissertation) Justus-Liebig Universität zu Giessen, pp. 52.

Van Es, L., and Schalk, A. F: 1914. Avian tuberculosis. N. Dak. Agr. Exper. Sta, Bul. 108.

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13

Infectious Coryza and Avian Mycoplasmosis*

Infectious Coryza (Roup, Hemophilus gallinarum Infection)

Infectious coryza is the name applied to a respiratory disease of chickens caused by the bacterium *Hemophilus gallinarum*. The disease is characterized by nasal discharge, frequent swelling or edema of the face, and sneezing. *H. gallinarum* infection was rather common during the 1930's and early 1940's and then almost disappeared, apparently occurring only in some flocks in California. The decreased incidence of the disease was attributed to better methods of isolation rearing, to the practice of disposing of the laying flock at the end of the year, and to the failure of *H. gallinarum* to survive outside the bird. However, the incidence of the disease appears to have increased some in recent years. It still is a problem in California and "Infectious Coryza" was reported as

being diagnosed in a few flocks in several states in 1963 (Angstrom, 1963, and Henderson, 1963), although definite confirmation of pathogenic *H. gallinarum* infection apparently has only been reported in California (Page, 1962a) and in Delaware (Benton, 1963) in recent years. It is also present in Brazil (Bueno, 1950), in Puerto Rico (Rivera-Analya *et al.*, 1953), and in Israel (Bornstein and Samberg, 1954).

Etiology. As early as 1927 de Blieck in Holland and Beach in the United States believed that the disease then known as contagious catarrh was a distinct entity. Since the disease frequently complicated fowl pox, one of the first problems was to separate the two infections. This was first accomplished by de Blieck (1931, 1932), who reported on the isolation of a pathogenic organism from a disease which he was able to transmit to pox-immune birds. He called the organism *Bacillus haemoglobinophilus coryzae gallinarum*. Nelson (1932) reported from New Jersey on the isolation of a hemophilic organism from uncomplicated coryza in chickens. Later

[405]

FIG. 13.1 — Hemophilus gallinarum in film of nasal exudate. ×810.
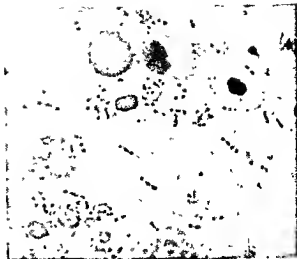
Schalm and Beach (1934), Eliot and Lewis (1934), and Delaplane *et al.* (1934) confirmed previous reports by de Blieck. Eliot and Lewis (1934) proposed the name *Hemophilus gallinarum*, by which the organism is known today.

H. gallinarum is a Gram-negative, pleomorphic, nonmotile bacterium. It is catalase negative, reduces nitrates, and ferments several carbohydrates under appropriate conditions (Page, 1962a). This organism, found in the sinus exudate of the infected chicken, has bipolar staining characteristics (Fig. 13 1). In young cultures it occurs as a short coccoid rod, but after 24 to 48 hours many long forms are found. The organisms form tiny, dewdroplike colonies on the surface of a suitable medium. *H. gallinarum* is fastidious in its growth requirements. Schalm and Beach (1936a, 1936b) found that their strains required two factors — an X-factor (hemin) which resisted autoclaving and was present in the red blood cells, and a V-factor (DPN) which resisted boiling for 5 minutes but was destroyed by autoclaving and was found in both the serum and red cells. Serum from defibrinated blood contained both factors. However, Gregory (1943) noted that the X-factor (hemin) was not necessary, and in more refined studies Page (1962a) demonstrated that *H. galli-*

narum readily grew in media which contained no iron porphyrin (hemin) derived from blood. He further found that reduced DPN was essential for growth and could be supplied by adding enzymatically reduced DPN by feeder (nurse) cultures of *Staphylococcus epidermidis*, or by the addition of chicken or sheep serum.

Most workers have considered that an atmosphere containing added CO_2 was necessary to obtain optimum growth of *H. gallinarum*. However, Nelson (1933b) obtained good growth on blood agar plates which were sealed with clay, and Page (1962a) recently demonstrated that *H. gallinarum* was not dependent upon CO_2 as such, but was microaerophilic. He obtained good growth with reduced oxygen levels as well as under completely anaerobic conditions. Methods to obtain reduced oxygen levels include the addition of CO_2 to the incubation chamber or the removal of some of the oxygen by burning out a candle in a sealed container.

Early workers devised a number of different media which supported the growth of *H. gallinarum*, due primarily to ingredients which supplied the necessary V-factor (DPN) although a source of the X-factor (hemin) was frequently included.

Nelson (1932, 1933a) and Delaplane *et al.* (1936) used blood at the base of agar

slants. Delaplane and Stuart (1939) found a growing yeast medium to be satisfactory. Cunningham and Stuart (1944) found an egg yolk medium suitable for growth of *H. gallinarum*. Gregory (1943) described a superior medium in which pieces of raw potato were used in chicken infusion broth. He emphasized the necessity of using salt in the medium. Hofstad (1959) used chicken infusion agar into which was incorporated 10 per cent chicken serum before slanting the agar. The organism colonized on the surface of the slant in a 10 per cent CO_2 atmosphere. This was a suitable medium for subculturing the organism after initial isolation on blood agar plates incubated in a partial atmosphere of CO_2. The use of blood agar plates incubated under a reduced oxygen tension appears to be a practical method for original isolation of *H. gallinarum*. Tryptose agar supplemented with a source of reduced DPN was found to be adequate by Page (1962a). Broth media are seldom employed for original isolation attempts because of the possibility of contaminant bacteria in respiratory tract exudates. However, Hofstad (1964) found that chicken meat infusion broth containing 3–5 per cent added chicken or turkey serum was valuable for subculturing *H. gallinarum* after original isolation. Page (1962a) obtained good growth when as little as 0.1 per cent chicken serum was added along with 1 per cent glucose to tryptose broth which had been filter sterilized after supporting the growth of *Staph. epidermidis*.

Another method for the propagation of *H. gallinarum* is the use of embryonated chicken eggs inoculated into either the yolk sac or the chorioallantoic sac. Infective yolk or allantoic fluid can then be harvested. Bacterial contaminants present in respiratory tract exudates limit the usefulness of this procedure for original isolation purposes.

H. gallinarum is an organism of low resistance outside the bird. De Blieck (1934) found it was killed after 24 hours at 37° C. when suspended in saline. The organism was dead when tested after 4 days at 22° C. Eliot and Lewis (1934) found it did not survive a temperature of 45° C. for 6 minutes when suspended in water. When suspended in hemolyzed blood broth, the organism was killed at 55° C. in 4 to 6 minutes, but survived 10 minutes at 50° C. Page (1962b) reported that the organisms in infectious nasal exudate suspended in tap water were viable for 3 but not 4 hours. Organisms which had been cultivated on agar medium and then suspended in tap water were nonviable within 4–12 minutes. *H. gallinarum* present on the legs of houseflies which had fed on infectious nasal exudate remained viable for only 15–30 minutes. Hofstad (1964) found the organism to survive for at least 10 years in the lyophilized state. The virulence of the organism for chickens is reduced by culturing on artificial media, and usually after 20 to 40 transfers the organism becomes avirulent. Recent reports by Page (1962a) and Page *et al.* (1963) indicate the existence of at least 2 serotypes of pathogenic *Hemophilus gallinarum*, although they were not found to be immunogenically distinct in cross protection studies. The 2 serotypes differed in their ability to ferment sucrose. He also described 3 nonpathogenic serotypes which were not typical of *H. gallinarum* (Page, 1962a). These organisms probably should only be designated as *Hemophilus* species at this time. They were strongly catalase positive, grew aerobically, required DPN (but not reduced DPN) for growth and some produced pigment in the presence of glucose.

Symptoms. In most cases of coryza there is involvement of the sinuses and nasal passages with a nasal discharge, swelling or edema of the face, conjunctivitis, and sneezing. Figure 13.2 illustrates the typical facial swelling. In males particularly there may be swollen wattles. Feed and water consumption by the affected flock usually is decreased, and in a laying flock this means fewer eggs, loss of weight, and increased number of culls. A foul odor may be detected in flocks where the disease has become chronic and complicated with

FIG. 13.2 — Artificial infection with infectious coryza showing facial edema.

other bacteria. Beach and Schalm (1936) and Delaplane and Stuart (1941a) have observed that *H gallinarum* infection also may involve the trachea and bronchi, causing rales and difficult breathing.

Transmission and incubation period. Infectious coryza most often occurs in the fall and winter. Outbreaks frequently are started by introducing carriers into the flock. Pullets may acquire the disease from recovered birds kept over from the previous year. Infection within the flock is spread by contact and by air-borne infected dust or droplets. The disease may spread rapidly through the flock, or transmission may take place more slowly depending, apparently, upon virulence of the organism and other factors. Page (1962b) concluded that the primary medium for intraflock transmission of infectious coryza was drinking water contaminated with infective nasal exudate. Intraflock transmission due to aerosol exposure appeared to be slower and less extensive in his studies. Transmission by caretakers and houseflies could not be demonstrated.

The incubation period after experimental inoculation is 18 to 36 hours. Susceptible birds exposed by contact to infected cases usually have symptoms of the disease in 1 to 3 days.

Course and mortality. The duration of infectious coryza is variable, but usually the disease persists through the cold months of the year if permitted to run its course. Complicating factors, such as secondary bacterial invaders, poor housing, parasitism, and inadequate nutrition, may add to the severity and duration of the disease. Nelson (1933b) has recorded a duration of 4 to 18 days in experimental cases produced by infected exudate. During the summer months infectious coryza may be less severe with a shorter duration. The virulence of various strains of the organism may alter the course of the disease. Delaplane *et al.* (1933) described a severe disease with high mortality in their original report on infectious coryza in Rhode Island. Schalm and Beach (1936a, 1936b) observed increased virulence by rapid passage of some strains in susceptible chicks and noted a difference in virulence on poultry farms.

Pathology. *H. gallinarum* produces an acute catarrhal inflammation of the mucous membranes of the nasal passages and sinuses. Airsacculitis was noted by Page (1962a,b). There is frequently a catarrhal conjunctivitis and a subcutaneous edema of the face and wattles. A chronic inflammatory process accompanied by caseous exudate in the sinuses, nasal passages, and conjunctival sacs is present in cases of long standing when the disease is complicated with other bacteria.

Diagnosis. A field diagnosis of *H. gallinarum* infection is difficult to make since other diseases, such as chronic cholera, fowl pox, A-avitaminosis, and the "chronic respiratory disease," may produce similar clinical symptoms. A method of making a field diagnosis was suggested by Delaplane and Stuart (1941b) and by Wernicoff and Goldhaft (1944). It consisted of treating the affected flock with sulfathiazole and observing the response. A diagnosis of *H. gallinarum* infection could be made if the flock responded favorably to the treatment within a week.

In the diagnostic laboratory it is desirable to inoculate susceptible chicks with suspected field material in order to de-

termine the transmissibility and the incubation period. If the inoculated chicks develop a nasal discharge and facial swelling within a few days, *H. gallinarum* infection may be suspected and attempts should be made to culture the organism, which may be found most consistently in the infraorbital sinus exudate. Isolation of an organism with characteristics of *H. gallinarum* and which will produce symptoms of coryza in chickens within a few days constitutes a diagnosis of infectious coryza.

Page (1962a) employed plate agglutination with hyperimmune chicken sera in his serological studies. It is possible that such a procedure might be employed with sera from convalescing birds to aid in making a laboratory diagnosis.

Treatment. Delaplane a n d Stuart (1941b) found sulfathiazole effective in reducing the severity of infectious coryza. Schlenker *et al.* (1941) found that concentrations of 3/16 gm., or greater, of sulfathiazole per ounce of mash established a blood level sufficient to prevent the development of symptoms of coryza after artificial exposure. Hamilton (1943) also found the drug to be effective in field outbreaks of the disease. Wernicoff and Goldhaft (1944) found sulfathiazole at the rate of ½ lb. per 100 lb. of dry mash to be effective in acute coryza. The above workers frequently noted a recurrence of the symptoms after the drug was withdrawn, thus indicating that it is necessary to continue or repeat the treatment. Improvement of the flock is usually observed within a week after treatment is begun. Streptomycin also has been found effective in treating infectious coryza (Bornstein and Samberg, 1954, 1955a, 1955b) at a dosage of 200 mg. intramuscularly. However, Page (1962b) found that his test isolate of *H. gallinarum* was relatively resistant to dihydrostreptomycin and sulfathiazole, but was reasonably sensitive to erythromycin and oxytetracycline. Intramuscular injections of erythromycin, oxytetracycline, and dihydrostreptomycin did not greatly alter the course of the disease in experimentally infected birds, though. Erythromycin given

for 4 days in the drinking water reduced the spread of infection among susceptible chickens, apparently due to its bactericidal effect in the water and in reducing the number of shedders, but following its removal a relapse in the flock was apparent. Modification of the feeding program to stimulate feed consumption may be desirable when birds are not eating. It is advisable to correct faulty ventilation or other factors contributing to the severity of the disease.

Eradication. The removal of recovered birds from the flock is necessary if the disease is to be eradicated from the premises, because recovered birds remain reservoirs of infection. The method of eradication depends upon circumstances on the farm —that is, size of flock, facilities, and purposes of the flock. The infected birds may be marketed at once and the premises cleaned before new chicks are brought on the place. Another more popular method is to treat the affected flock and keep it isolated until new stock has been raised in isolation as replacements. After the infected or recovered birds are marketed, the house should be cleaned and disinfected before housing the new stock.

Prevention. Because recovered birds are carriers and are responsible for new outbreaks, such practices as buying breeding males or started chicks should be discouraged. Only day-old chicks should be secured for replacement purposes unless the source is known to be free of infectious respiratory disease. Isolation rearing and housing away from old stock are desirable practices for the prevention of respiratory diseases.

A more recent approach to the prevention of infectious coryza consists of various "vaccination" procedures. Tennison and Siddle (1961) inoculated young chickens intramuscularly with live *H. gallinarum* of embryo origin. They reported reasonable protection when the birds were challenged 3–4 weeks later. However, live cultures should be employed judiciously if eventual eradication of the disease is contemplated. They also conducted trials

with a formalin inactivated bacterin which produced reasonable protection following 2 doses given 2 weeks apart. Clark and Godfrey (1961) used formalinized embryo fluids to prepare an oil emulsion type bacterin. They noted a decrease in the severity and duration of coryza in flocks which were naturally exposed to chickens with infectious coryza, CRD, and pasteurellosis.

Page et al. (1963) also studied the use of formalinized bacterins. They noted only limited resistance to upper respiratory infection, but did demonstrate some protection against the occurrence of airsacculitis and a resistance to a drop in egg production in laying chickens challenged with *H. gallinarum*. The effectiveness of bacterins under various field conditions needs further study, but undoubtedly will vary with the extent of exposure to natural infection and complicating conditions such as pasteurellosis, CRD, and other respiratory infections.

REFERENCES

Angstrom, C. I: 1963. Report of the committee on nomenclature and reporting of disease. Northeastern Conference on Avian Diseases, June 1963. Avian Dis. 7:509.

Beach, J. R., and Schalm, O. W: 1936. Studies of the clinical manifestations and transmissibility of infectious coryza of chickens. Poultry Sci. 15:466.

Benton, W J: 1963. Personal communication.

Bornstein, S, and Samberg, Y: 1954. The therapeutic effect of streptomycin on infectious coryza of chickens caused by *Hemophilus gallinarum*. II. Isolation and culture of *H. gallinarum*, and some of its biochemical reactions Am. Jour. Vet. Res. 15,612.

———, and Samberg, Y: 1955a. The therapeutic effect of streptomycin on infectious coryza of chickens caused by Hemophilus gallinarum. III. In vitro and in vivo sensitivity of *Hemophilus gallinarum* to streptomycin Am Jour. Vet. Res. 16,321.

———, and Samberg, Y: 1955b. The therapeutic effect of streptomycin on infectious coryza of chickens caused by *H. gallinarum*. V. The effect of parenteral streptomycin in high dosage on egg production and growth in birds affected with infectious coryza. Poultry Sci. 34.896.

Bueno, R. C.: 1950. A coriza das aves. (Coryza of fowl) Biologico (São Paulo) 16:223.

Clark, D. S., and Godfrey, J. F.: 1961. Studies of an inactivated *Hemophilus gallinarum* vaccine for immunization of chickens against infectious coryza Avian Dis. 5:37.

Cunningham, C. H , and Stuart, H. O.: 1944. Egg-yolk medium for the cultivation of *Hemophilus gallinarum*. Am. Jour. Vet. Res. 5.142.

de Blieck, L.: 1931. En haemoglobinophille bacterie als oorzaak van coryza infectiosa gallinarum. Tijdschr. voor Diergeneesk 58.510.

———: 1932. A hemoglobinophilic bacterium as the cause of contagious catarrh of the fowl. Vet. Jour. (London) 88:9

———: 1934. *Coryza infectiosa gallinarum*. Proc. 12th Internat Vet. Cong. 3:161.

Delaplane, J P., Erwin, L. E., and Stuart, H. O.: 1936. The isolation of hemophilic bacillus in pure culture and the reaction of chickens to extranasal inoculation thereof. Jour. Agr. Res 52.277.

———, Erwin, L. E., and Stuart, H. O., 1934. A hemophilic bacillus as the cause of an infectious rhinitis. R I. Agr. Exper. Sta., Bul 244:1.

———, and Stuart, H. O · 1939. A growing yeast medium for the cultivation of hemophilic bacilli. Jour. Am. Vet. Med. Assn. 95:326

———, and Stuart, H. O 1941a. An atypical type of *H. gallinarum* (infectious coryza) infection in chickens, Jour. Am. Vet. Med. Assn. 98-501.

———, and Stuart, H O · 1941b. The chemotherapeutic value of sulfathiazole in preventing and treating infectious coryza (*Hemophilus gallinarum* infection) in chickens. Jour. Am. Vet. Med. Assn. 99 41

———, Stuart, H. O., and Bunyea, H.: 1933. A preliminary report of an apparently new respiratory disease of chickens. Jour. Am. Vet. Med. Assn. 82:772.

Eliot, C. P., and Lewis, M. K: 1934. A hemophilic bacillus as a cause of infectious coryza in the fowl. Jour. Am. Vet. Med. Assn. 84:878.

Gregory, D. W.: 1943. A superior medium for the hemophilic fowl coryza bacillus. Am. Jour Vet. Res. 4:32.

Hamilton, C. M.: 1943 Treatment of infectious coryza in chickens with sulfathiazole. Jour. Am. Vet. Med. Assn. 103:144

Henderson, W.: 1963. Summary of poultry diseases reported at the North Central Poultry Disease Conference. 1963. Avian Dis. 7:516.

Hofstad, M. S.: 1959. Infectious coryza. In Biester, H. E., and Schwarte, L. H., eds., Diseases of Poultry, 4th ed. Iowa State University Press, Ames, Iowa. P. 317.

———: 1964. Personal communication.

Nelson, J. B.: 1932. Etiology of an uncomplicated coryza in the domestic fowl. Proc. Soc. Exper. Biol. and Med. 30:306.

———: 1933a. Studies on an uncomplicated coryza in the domestic fowl: 1. Isolation of a bacillus which produces a nasal discharge. Jour. Exper. Med. 58:289.

———: 1933b. Studies on an uncomplicated coryza in the domesticated fowl: 11. The relation of the bacillary coryza to that produced by exudate. Jour. Exper. Med. 58:297.

Page, L. A.: 1962a. Haemophilus infections in chickens. I. Characteristics of 12 Haemophilus isolates recovered from diseased chickens. Am. Jour. Vet. Res. 23:85.

———: 1962b. Haemophilus infections in chickens. III. Factors in intraflock transmission of infectious coryza and its chemical and antibiotic therapeusis. Avian Dis. 6 211.

Rosenwald, A. S., and Price, F. C.: 1963. Haemophilus infections in chickens. IV. Results of laboratory and field trials of formalinized bacterins for the prevention of disease caused by Haemophilus gallinarum. Avian Dis. 7:239.

Rivera-Analya, J. D., Martinez de Jesus, J., and Orlando, R.: 1953. The use of streptomycin (dihydrostreptomycin sulfate) in the treatment of fowl coryza or "moquillo." Jour. Agr. Univ. Puerto Rico 37:52.

Schalm, O. W., and Beach, J. R.: 1934. The etiology of a respiratory disease of chickens. Science 79:416.

———, and Beach, J. R.: 1936a. Cultural requirements of the fowl coryza bacillus. Jour. Bact. 31:161.

———, and Beach, J. R.: 1936b. Studies of infectious coryza of chickens with special reference to its etiology. Poultry Sci. 15:473.

Schlenker, F. S., Delaplane, J. P., and Stuart, H. O.: 1941. The chemotherapy of infectious coryza with sulfathiazole: Correlation between feeding levels and blood concentrations. Am. Jour. Vet. Res. 2:443.

Tennison, L. B., and Siddle, P. J.: 1961. Limited study with Haemophilus cultures. Avian Dis. 5:352.

Wernicoff, N. E., and Goldhaft, T. M.: 1944. The field use of sulfathiazole in some diseases of poultry. Cornell Vet. 34:199.

Avian Mycoplasmosis

(Mycoplasma gallisepticum infection, chronic respiratory disease)

Avian mycoplasmosis essentially is the disease caused by *Mycoplasma gallisepticum* infection, which frequently has been called chronic respiratory disease (CRD) of chickens and infectious sinusitis of turkeys. It is characterized by respiratory rales, coughing, and nasal discharge. The clinical manifestations are slow to develop and the disease has a long course. The disease has become an important flock problem in all areas of the United States. It is also present in Canada (Fahey *et al.*, 1953), Holland (de Blieck, 1950), Brazil (Garust and Nóbrega, 1956), the Philippines (Quizon, 1958), India (Pathak and Singh, 1961), Japan (Tajima *et al.*, 1958), England (Chu, 1958), Germany (Hartwigk, 1958), Switzerland (Keller, 1958), Egypt (Eissa, 1956), France (Brion *et al.*, 1958), Australia (Cottew, 1956), South Africa (Coles and Cumming, 1959) and Czechoslovakia (Striker and Fisera, 1955).

Etiology. Nelson (1936a-d, 1938, 1939) studied an agent, isolated from a coryza

of chickens, which he described as coccobacilliform bodies. He was able to grow them in tissue culture, embryonating chicken eggs, and in cell-free medium. Smith *et al.* (1948) studied Nelson's agent and found it grew well in ascitic peptic digest plates and in infusion broth enriched with 30 per cent horse serum. From cultural and morphological characteristics they concluded that Nelson's coccobacilliform bodies could be included in the pleuropneumonia group of organisms.

Delaplane and Stuart (1943) cultivated an agent in embryos isolated from chickens with a chronic respiratory disease, and later from turkeys with sinusitis (Delaplane, 1949). Markham and Wong (1952) placed the agent of chronic respiratory disease in the pleuropneumonia group. It appears likely that Nelson's agent was the same as the one later isolated by Delaplane. This is further supported by the finding of Adler and Yamamoto (1956b) that a combination of *Hemophilus gallinarum* and avian pleuropneumonialike organism

FIG. 13.3 — Giemsa's stained smear of sedimented broth culture of Mycoplasma gallisepticum, ×885.

(PPLO) resulted in a disease of rapid onset and long duration which Nelson produced with a combination of *H. gallinarum* and coccobacilliform bodies.

Edward and Kanarek (1960) proposed the name *Mycoplasma gallisepticum* for the typical pathogenic PPLO which causes chronic respiratory disease in chickens and infectious sinusitis of turkeys. The earlier named species *M. gallinarum* (Freundt, 1957) was found to represent a nonpathogenic serotype.

The organism is usually coccoid and about 0.5μ in size (Fig. 13.3). It has been studied by electron microscopy, and some variation in morphology has been found (Reagan *et al.*, 1953; White *et al.*, 1954). Some strains are spherical and others contain filaments (Fig. 13.4). Shifrine *et al.* (1962) studied the edge of growing colonies and concluded that the elementary cells were hexagonal and originated from within larger cells or by fragmentation of peripheral filaments. The organism stains well with Giemsa's stain and requires for its growth a medium enriched with serum or serum fraction. It is resistant to penicillin, polymyxin, neomycin (Wong and James, 1953), and low concentrations (1:4000) of thallous acetate. It can be grown on serum-enriched agar medium where it produces a characteristic colony with a dense, raised, central area (Fig. 13.5). Most strains of *M. gallisepticum* will cause agglutination of washed red blood cells of the chicken and turkey. Another
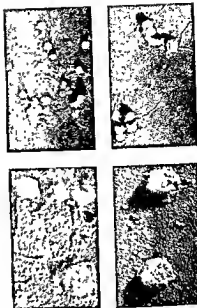
FIG. 13.4 — (Top left) Electron micrographs of M. gallisepticum Isolate 595. ×3,500. (Top right) Same. ×5,000. (Bottom left) Same. ×11,000. (Bottom right) Isolate 197. ×14,000. (Corr and Hofstad.)

characteristic of the organism is its ability to pass through filters which ordinarily retain bacteria. Using embryo-propagated material in the form of a suspension of the chorioallantoic membrane at a 1:100 dilution, the organism filters through the Selas 02, Berkefeld V and N, and Mandler 6 and 7 filters if sufficient amounts are filtered. It is withheld by the Berkefeld W, and the Seitz sterilizing pad. Organisms in broth culture readily pass through the Selas 02, but are partially retained by the 03 Selas filter.

Mycoplasma gallisepticum can be propagated in embryonating chicken eggs where it may or may not cause death of the embryo. Some strains, after a few passages, regularly cause mortality when inoculated into the yolk sac of seven-day-old embryonating chicken eggs (Hoyt *et al.*, 1951; Hofstad, 1952). On original isolation of some strains in embryos, there may be no mortality and no definite lesions, and it is necessary to make additional passages or to culture the embryo in suitable broth
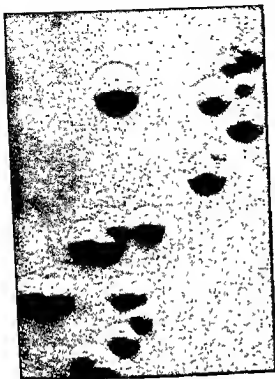
FIG. 13.5 — Colonies of Mycoplasma gallisepticum on 20 per cent chicken serum agar plate. X40.

FIG. 13.6 — Joint involvement and stunting of 18-day-old chicken embryos inoculated with strain 640 of Mycoplasma E serotype at 7 days into the yolk sac.

in order to identify the organism. Some strains may produce caseous exudation in the respiratory tract of embryos (Van Roekel et al., 1952) but dwarfing, generalized edema, liver necrosis, and enlarged spleen in infected embryos dying between 14 and 21 days are most commonly observed (Chute and Cole, 1954).

Delaplane (1948) observed "joint abscesses" in infected embryos (Fig. 13.6) as did Van Roekel et al. (1957), Thompson (1954), Moulton and Adler (1957), Calnek and Levine (1957), and Chute (1960) who described the lesions as being primarily subcutaneous periarticular granulomas with necrotic centers and a border of epithelioid cells, some of which had coalesced to form giant cells. However, Yoder and Hofstad (1964a) concluded that such lesions were produced by Mycoplasma representing other serotypes than that of M. gallisepticum.

The organism reaches its highest concentration in the yolk sac and yolk and in the chorioallantoic membrane just prior to death of the embryo (Hofstad, 1952; Luginbuhl and Jungherr, 1953).

A number of media have been used for isolation and growth of M. gallisepticum (Markham and Wong, 1952; Grumbles et al., 1953; Adler and Yamamoto, 1956c; Hofstad and Doerr, 1956; Fabricant, 1959). All these have as enrichment either a concentrated serum fraction (Difco) or heat-inactivated horse, avian, or swine serum. Comparisons of different media have been made (Adler et al., 1954; Lecce and Sperling, 1954; Taylor et al., 1957; Taylor and Fabricant, 1957; Fabricant, 1958 and 1959; Adler and Berg, 1960). It is apparent that several types of liquid or agar media will support the growth of Mycoplasma of avian origin, but it is doubtful if any single medium is superior for the isolation of all possible Mycoplasma.

M. gallisepticum ferments dextrose and

maltose with the production of acid, but not gas. It does not ferment lactose. The fermentation of sucrose has been reported by several investigators (Grumbles et al., 1953; Granforte et al., 1955; Adler et al., 1958; Yamamoto and Adler, 1958b; and Kleckner, 1960). However, Yoder and Hofstad (1961a) were unable to demonstrate the fermentation of sucrose by any of the 29 isolates of M gallisepticum which they studied. Results with trehalose, mannitol, and galactose have also been variable

Granforte et al. (1955) studied seven isolates of avian Mycoplasma from different geographic areas and found these to be serologically identical and with similar hemagglutinating capacities. However, Adler et al (1957) differentiated at least 2 serotypes. Five serotypes were apparent as reported by Adler et al (1958) and were subsequently further characterized by Yamamoto and Adler (1958a, 1958b). Kleckner (1960) described 8 serotypes designated A through H. Serotype A was represented by M gallisepticum, which has frequently been designated as the S6 serotype (Zander, 1961) Yoder and Hofstad (1962) characterized another serotype. All of these reported serotypes were included in the 12 serotypes (A through L) which were recently characterized by Yoder and Hofstad (1961a) and in the 19 serotypes (A through S) characterized by Dierks (1963) Fabricant (1960), Fabricant and Levine (1962), and Kelton and Van Roekel (1963) employed the colony inhibition technique to differentiate various serotypes. They both noted that isolates representative of serotypes E and G were indistinguishable by the colony inhibition technique although separable by agglutination assays. Adler et al. (1961) described a saprophytic Mycoplasma obtained from chickens which was subsequently identified as M. laidlawii (Adler, 1964). Thus, there have been at least 20 serotypes of Mycoplasma found to be associated primarily with the respiratory tract of various avian species. However, Mycoplasma gallisepticum is of major significance as a pathogen for chickens.

M. gallisepticum is well preserved in lyo-

philized form and also in the frozen state. Infective turbinates suspended in tryptose phosphate broth have remained infective for five years stored at approximately −30° C. Yoder and Hofstad (1961a) found broth cultures to be viable after at least 4 years storage at −30°C. They also obtained viable M. gallisepticum from lyophilized chicken turbinate suspensions which had been stored at 4° C. for approximately 14 years. Fabricant (1953) recovered the organism from 60 per cent, 35 per cent, and 13 per cent of infective materials stored at −25°C. for one, two, and three years, respectively. Broth suspensions of infective chorioallantoic membranes lost their infectivity after 1 hour of exposure to 45° C., or after 20 minutes of exposure to 50° C. Similar material lost its infectivity by the third week in the refrigerator at 5° C. Olesiuk and Van Roekel (1952), however, found infective allantoic fluid to remain infective for 4 days in the incubator, for 6 days at room temperature, and 32 to 60 days in the refrigerator.

While M. gallisepticum is considered the primary cause of chronic respiratory disease, other organisms frequently cause complications, particularly in broilers (Baddle and Cover, 1957). Escherichia coli (especially O-group 2) has been found to be a frequent complicating organism in cases of air-sac infection in broilers (Wasserman et al., 1954; Gross, 1956; Glantz et al., 1962). Newcastle disease or infectious bronchitis infection may precipitate outbreaks of M. gallisepticum infection (Van Roekel et al., 1957). The effect of M. gallisepticum, E. coli and infectious bronchitis virus in singly and in multiply infected chickens was studied by Gross (1961a) and Fabricant and Levine (1962). They reproduced a severe air-sac infection, frequently designated complicated CRD or air-sac infection, when all 3 agents were combined. They further noted that E. coli could not readily infect the air sacs unless they were previously invaded by M. gallisepticum alone or in combination with either infectious bronchitis virus or (Gross, 1961b,

1962) Newcastle disease virus. Adler et al. (1962) and Blake (1962) noted increased severity and duration of the disease when both M. gallisepticum and infectious bronchitis virus were present. A virus isolated by Fahey and Crawley (1954a) from outbreaks of chronic respiratory disease has not been proved to have a significant role in the etiology of the disease.

It has been postulated by Kelton and Gentry (1957 and 1960) that the avian pleuropneumonialike organisms (PPLO) associated with chronic respiratory disease of chickens are L-forms of certain bacteria. This is based on the observation that PPLO broth cultures presumably revert primarily to staphylococci by the third transfer. However, conclusive proof that avian PPLO are actually L-forms of bacteria has not been published.

Transmission and incubation period. Outbreaks of the disease are often started by carriers, and the disease is spread by contact and by air-borne dust or droplets (Fahey and Crawley, 1955a). Van Roekel et al. (1952) found evidence that the disease may be transmitted through the egg. This has been confirmed by other investigators (Fahey and Crawley, 1954b; Cover and Waller, 1954; Hofstad, 1957a). The isolation of M. gallisepticum from the oviduct of infected hens and from the semen of infected roosters was reported by Yoder and Hofstad (1964a). Delaplane and Stuart (1943) and Van Roekel et al. (1952) found the incubation period to vary from 4 to 21 days in experimental transmission. Hofstad (1952) found that 65 per cent of 233 chicks had symptoms of nasal discharge between 11 and 18 days following intranasal inoculation of infective turbinate suspensions.

Susceptible hosts. The disease is common in chickens and turkeys. Guinea fowl and pheasants were readily infected with M. gallisepticum (Van Roekel and Olesiuk, 1953), and Osborn and Pomeroy (1958) isolated the agent from naturally infected pheasants. Pigeons and partridges were included as susceptible hosts by Jungherr et al. (1953). Natural infection in chukar

partridges (Alectoris graeca) has been reported by Wichmann (1957) and Yoder and Hofstad (1964a). The later workers were unable to produce infection in pigeons or Bobwhite quail (Corlinus virginianus) although previous reports suggested the infection had occurred in pigeons (Winterfield, 1953; Gianforte et al., 1955). Wills (1955) isolated the agent from an infected peacock (Pavo cristatus).

Symptoms. The most characteristic signs of the natural disease in adult flocks are tracheal rales, nasal discharge, and coughing. Feed consumption is reduced and the birds lose weight. In laying flocks egg production declines but is usually maintained at a lowered level. Male birds frequently have the most pronounced symptoms, and the disease is most severe in the winter. In broiler flocks most outbreaks occur between 4 and 8 weeks of age. The symptoms are frequently more marked than observed in mature flocks. The severe outbreaks observed in broilers are frequently due to complications (see Etiology.)

Course and mortality. The duration of M. gallisepticum infection is long, and the severity may vary considerably in different flocks. Inbred lines of chickens often have a more severe form of the disease with a longer duration than crossbred chickens. When the disease occurs during the summer in growing flocks, the birds usually recover more promptly than those which acquire the disease during the cold months of the year. Delaplane and Stuart (1943) observed the duration of experimental cases to vary from 21 to 68 days.

The mortality is negligible in adult flocks, but there may be considerable loss from lowered egg production and development of culls. In broilers, the mortality varies from very low in the uncomplicated disease to as much as 30 per cent in the complicated outbreaks. Retarded growth, grading down of carcasses, and condemnations constitute additional loss in broilers.

Pathology. The gross lesions consist primarily of catarrhal exudate in the nasal passages, trachea, bronchi, and air sacs.

FIG. 13.7 — lympho-follicular or "beading" reaction in air sac of experimentally inoculated turkey. (Van Roekel et al., Univ. of Mass.)

The air sacs frequently contain caseous exudate and may present a "beaded" appearance (Fig. 13.7). The tracheal mucosa is usually thickened. Some degree of pneumonia may be observed (Van Roekel et al., 1952).

The microscopic pathology has been studied by Jungherr et al. (1953) and by Van Roekel et al. (1957). They found marked thickening of the mucous membrane of the affected tissues due to infiltration with mononuclear cells and hyperplasia of the mucous glands. Focal areas of lymphoid hyperplasia (lympho follicular reaction) were commonly found in the submucosa (Figs. 13.8, 13.9, 13.10). Johnson (1954) regarded these as specific for chronic respiratory disease. Hitchner (1949) found them characteristic in his study of the pathology of infectious sinusitis of turkeys. However, Barber (1962) observed similar lesions in apparently normal turkeys, and suggested that the presence of lympho follicular lesions may be of limited diagnostic value. In the lungs, in addition to pneumonic areas and the lympho follicular changes, granulomatous lesions were also found. Van Roekel et al. (1957) found granulomatous lesions in lungs of about 22 per cent of field cases in chickens but in only 7 per cent of experimentally inoculated chickens.

Diagnosis. The diagnosis of $M.$ $gallisepticum$ infection should be based on isolation and identification of the organism. Suspensions of tracheal or air-sac exudate, turbinates, or lungs may be cultured directly in a suitable broth medium. Contamination may be controlled by a 1:4000 dilution of thallous acetate and up to 2,500 U. per ml. of penicillin. Contaminants may also be eliminated by filtering the suspension through a series of Selas filters beginning with the 01 and continuing through the 015 and then through the 02 filter. Both the 015 and the 02 filtrates should be cultured. The advantage of fil-
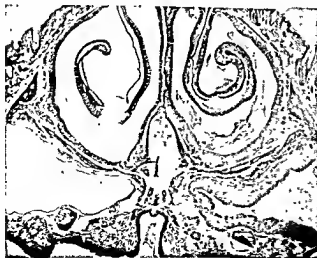
FIG. 13.8 — Section through nasal passages and sinuses of experimental chicken. Unilateral mucosae thickening of sinus and nasal passage. X6. (Van Roekel et al., Univ. of Mass.)

tration is that it eliminates tissue debris in the cultures, and the material is then suitable for embryo inoculation. The disadvantages are that it is time-consuming, and the possibility exists that small amounts of the organism may be adsorbed to the filter. Contaminants may also be controlled by first inoculating the material into the sinus of a turkey. Usually the sinus exudate that results is free of contaminants.

Cultures should be incubated several days to a week, and if tissue debris is present, the culture is shaken, allowed to settle a few minutes, and a transfer made to fresh medium. Growth may be detected grossly or an indicator system may be used, such as phenol red with maltose or triphenyltetrazolium chloride (Somerson and Morton, 1953). The latter is reduced by *M. gallisepticum*, changing the broth to pink or red, and has been found useful by Yoder and Hofstad (1964). Smears made from the sediment after centrifugation of cultures at 2,500 RPM for 15 minutes and stained with Giemsa's stain (2 ml. concentrated stain to 50 ml. water) should reveal small coccoid organisms, as shown in Fig-

FIG. 13.9 — Section of sinus in thicken. Subepithelial infiltration of mononuclear cells and lympho-follicular reaction. ×36. (Van Roekel et al., Univ. of Mass.)
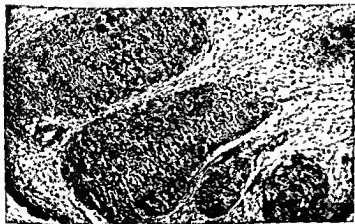
FIG. 13.10 — Air sac of 7-week-old experimental chicken, lympho-follicular reaction. ×100. (Van Roekel et al., Univ. of Mass.)

ure 13.3. Frequently the organisms appear in large masses or clumps in the first few culture passages. Enriched agar medium may be inoculated from the broth culture to study the colonial morphology.

Further identification of *M. gallisepticum* can be made by preparing an antigen for an agglutination plate test with a known positive agglutinating serum. The antigen may also be used to prepare, in chickens, turkeys, or rabbits, an antiserum which is then tested against a known culture of *M. gallisepticum*. The hemagglutinating capacity of the isolate should be tested, and if positive, a hemagglutination-inhibition test may be set up with known positive serum. It is desirable to make a pathogenicity test either in the sinuses or air sacs of turkeys or in chickens. Evidence that the organism is pathogenic may be manifested if such an inoculation results in sinus swelling, air-sac exudate, and antibody response to a known antigen of *M. gallisepticum*. Histological sections would also aid in the diagnosis (see Pathology).

Serological procedures are available to aid in the diagnosis. However, since these tests are only evidence of recent or past infection, they cannot be relied upon solely for a diagnosis. A positive serological test, together with history and symptoms typical of the disease, would constitute a presumptive diagnosis.

Treatment. *M. gallisepticum* is susceptible to contact with certain antibiotics: streptomycin, oxytetracycline, chlortetracycline, erythromycin, magnamycin, spiramycin, and tylosin (Wong and James, 1953; Domermuth and Johnson, 1955; Yamamoto and Adler, 1956; Hamdy et al., 1957; Kiser et al., 1961; Yoder et al., 1961). However, some strains of *M. gallisepticum* have been reported to be rather resistant to streptomycin, erythromycin, and spiramycin (Fahey, 1957; Yoder et al., 1961; Domermuth, 1958, 1960; Kiser et al., 1961).

Various antibiotics and chemicals have been injected or administered in the feed or water for the treatment of chronic respiratory disease (Peterson, 1953; Carson et al., 1954; White-Stevens and Zeibel, 1954; Cover, 1955; Fahey and Crawley, 1955b; Lecce and Sperling. 1955; Adler et al., 1956; Barnes et al., 1960, 1961; Gross, 1961b; Heishman et al., 1960, 1962; Kiser et al., 1960; Olesiuk and Van Roekel, 1959; Olesiuk et al., 1957, 1964; Olson et al., 1959, 1960). The results of the various treatment studies have been variable, probably reflecting the varied complicating infections present in a wide spectrum of age groups under diverse conditions. In many cases it is doubtful if the small increase in weight gains or egg production and moderate reduction of carcass condemnations is sufficient to cover the cost of treatment. However, some of the more commonly employed treatments which tend to provide favorable results include the use of oxytetracycline or chlortetracycline at 200–400 grams per ton of feed for at least several days. Such broad spectrum antibiotics have been potentiated with approximately 0.5 per cent terephthalic acid and sometimes a reduced calcium ration. Tylosin has been injected subcutaneously at 3–5 mg. per pound of body weight or administered at 2–3 gm. per gallon of drinking water for 3–5 days. Antibiotic injections of infected breeding stock to control egg transmission have been widely used (see Control).

Immunity. Chickens which have recovered from clinical signs of the disease have some degree of immunity. Such flocks, however, carry the organism and can transmit the disease to susceptible stock by contact or can infect the progeny of the flock by way of the egg. (For immunization see Prevention and control.)

Serological procedures. Jungherr et al. (1953) first recognized the possibilities of certain serological procedures in *M. gallisepticum* infection. The rapid serum plate test was used by Adler (1954). Antigen is prepared according to the procedure of Adler and Yamamoto (1956a) or Hofstad and Doerr (1956) or Hall (1962). The test is performed by placing a drop of serum on a white porcelain or glass plate and mixing with a drop of stained anti-

gen. After the drops are mixed to make a spot about 2 cm. in diameter, the plate is rotated gently and the test read within 2 minutes. Turkey serum reacts more slowly than chicken serum in the test.

The tube agglutination test was found more reliable than the plate test by Jungherr et al. (1955). It was also found more reliable than the plate test in testing turkey serum (Hofstad, 1957b). The antigen prepared for the plate test can be used in the tube test when diluted 1:20 in phenolized (0.25 per cent) buffered saline (pH 7). For routine flock testing, it is suggested that the 1:12.5 dilution (.08 ml. serum plus 1 ml. antigen) be used. The test is read after overnight incubation at 37° C. Clearing of the supernatant fluid, with clumps of antigen covering the entire bottom of the tube, is indicative of a positive test.

The hemagglutination-inhibition (HI) test has been used by Fahey (1954), Fahey and Crawley (1954b), Crawley and Fahey (1957), Hall et al. (1961), Hofstad (1957b), and Yoder and Hofstad (1964a). The procedure of the HI test is essentially the same as the one used in Newcastle disease. The antigen used may be prepared by growing a suitable hemagglutinating strain of M. gallisepticum in broth. The antigen should be harvested at the peak of hemagglutinating (HA) activity. The organism is centrifuged out at 4,500 RPM for 1 hour in a refrigerated centrifuge. It is then suspended in 50 per cent glycerin and stored in the freezer. The HA titer is determined by making two-fold dilutions in 0.5-ml. amounts of buffered saline (pH 7) containing a 1:1000 dilution of normal serum. Washed 0.25 per cent chicken or turkey red blood cells are added in 0.5-ml. amounts to each dilution of the antigen. The test is read when the cell controls have sedimented, usually after 1 hour at room temperature. Agglutination is recognized by a uniform layer of cells covering the entire bottom of the tube, while in the negative tube a button of cells is found in the center of the bottom (Fig. 13.11). No elution occurs, so the test can be read at any time after 1 hour. If turkey cells are used, usually a two-fold higher titer is obtained than with chicken cells.
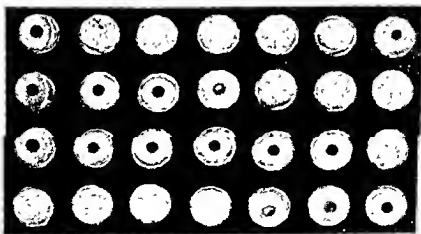
FIG. 13.11 — Hemagglutination-inhibition (HI) test. (Bottom row) Titration of antigen (M. gallisepticum). From left to right 4 tubes show agglutination of red cells; tube 5 is intermediate; tubes 6 and 7 show normal settling of red cells. (Top row) Negative serum in HI test. Tube 1 is serum control; tubes 2 through 5 show agglutination of cells; tube 6 is a saline blank with no red cells; tube 7 is cell control. (Second row from top) Positive serum-low titer; tube 1 is serum control; tubes 2 and 3 show complete inhibition; tube 4 shows partial inhibition; tubes 5-7 show no inhibition. (Third row from top) Positive serum — higher titers inhibition through tube 6.

The HI procedure can be used for both chicken or turkey serum When testing chicken serum, 4 HA units are used in the test, while 2 HA units can be used for turkey serum One HA unit is contained in the highest dilution of antigen which causes complete agglutination of 0 5 ml of 0.25 per cent red blood cells To set up the test, a series of 10 tubes are placed in a rack for each chicken serum sample to be tested. To tube 1 is added 0 8 ml of diluent, to tube 2 is added 0 5 ml of antigen containing 8 HA units, and to tubes 3 through 10 is added 0 5 ml of antigen containing 4 HA units Using a 1 ml. pipette, 0.2 ml of serum is removed from the serum sample and placed in tube 1 to make a 1·5 dilution of serum Tube 1 becomes the serum control The contents of tube 1 are mixed with the pipette, and a 0.5-ml. amount is transferred to tube 2 Using the same pipette, two-fold dilutions are continued through tube 10, discarding 0.5 ml from tube 10 after mixing has been done. Negative and positive serums should be included as controls A 0.5-ml. amount of 0.25 per cent red cell suspension is then added to each tube; the rack is shaken and set at room temperature for 1 hour after which the test can be read. A positive serum will inhibit agglutination to a certain dilution of serum. The results may be recorded as HI units (highest dilution of serum in which there is complete inhibition × the HA units used). For example, if the end point of inhibition is the 1:320 dilution, the serum would contain 320 × 4, or 1,280 HI units.

Prevention and control. Strict isolation of the flock should be maintained to avoid introduction of the disease into a clean flock. Breeding males should not be added to a flock unless they are from clean flocks as determined by negative serological tests for evidence of chronic respiratory disease. Replacements should be by day-old chicks originating from breeder flocks found free of chronic respiratory disease by serological tests.

Various inactivated vaccine preparations were reported to be of little value in pre-

venting M. gallisepticum infection by Prier and Dart (1951) and Adler et al. (1960). Attempts to immunize young birds with infective M. gallisepticum preparations inoculated intramuscularly have not been very satisfactory (Adler et al., 1960, and Donnermuth, 1962). Infective preparations inoculated via the respiratory route gave somewhat better protection against subsequent challenge (McMartin and Adler, 1961) and has been explored further to determine its effect on egg transmission of M. gallisepticum to their progeny (Olson et al., 1962, 1961, and Fabricant and Levine, 1963). The possible benefit of some reduction of egg transmission must be evaluated in the light of the hazard of perpetuation of the disease and the production of flocks which will be reactors when tested by the serologic procedures employed with most other control efforts.

Elimination of the disease in infected breeding flocks is a difficult problem, particularly when the flocks cannot be disposed of because of valuable inbred lines and families. One method is the hatching of small groups of a few hundred chicks in isolation and separating the clean groups from the infected ones by serological procedures. This procedure has been tried with some success by Dunlop and Strout (1956). The procedure is difficult to follow for most large breeding establishments where isolation areas are not available.

Attempts to eliminate egg transmission of the infection by monthly intramuscular injections of 200 mg. of streptomycin and dihydrostreptomycin in infected breeder flocks (Crawley and Fahey, 1955; Adler et al., 1956; Von Roekel et al., 1958) have not been found to be consistently effective. The use of other antibiotics for this purpose is currently being evaluated.

The dipping of warm hatching eggs in cold solutions of antibiotics, especially erythromycin and tylosin at approximately 400–1000 p.p.m., has been investigated as a means of reducing egg transmission of M. gallisepticum due to the effects of the absorbed antibiotic (Chalquest and Fabri-

cant, 1959; Levine and Fabricant, 1962; Olson et al., 1962; Stuart and Bruins, 1963; Hall et al., 1963; Yoder and Hofstad, 1964b). Alls et al. (1963, 1964) reported on the various factors affecting the permeability of dipped eggs and on the mechanics of the dipping process.

In general the dipping process has reduced, but not completely eliminated, the possibility of egg transmission. The influence on hatchability has not been consistently favorable, and the problem of bacterial contamination of dipped eggs has frequently been significant. The process is not recommended for widespread routine use at this time.

Although no consistently effective procedure has been developed for the control of M. gallisepticum infection in chickens, the application of one or more of the currently available procedures may lead to the establishment of clean replacement breeders. Strict attention must be given to sanitation and isolation procedures. The serological tests offer considerable aid in determining the M. gallisepticum status of flocks at various times, but cannot be used to eliminate infection from a flock by the testing and elimination of individual reactor birds, such as those used in the control of pullorum disease. Repeated random testing of 5–10 per cent of the birds in a flock may be adequate for preliminary investigations, but the testing of 100 per cent of the chickens in a flock near the time of sexual maturity probably is necessary to establish the status of breeder flocks. Moulthrop (1962) reported on the favorable performance of broiler chicks raised from M. gallisepticum-free breeder stock.

REFERENCES

Adler, H. E.: 1954. A rapid slide agglutination test for the diagnosis of chronic respiratory disease in the field and in laboratory-infected chickens and turkeys. Proc. 90th Ann. Meet. Am. Vet. Med. Assn., p. 346.

———: 1964. Mycoplasma inocuum is M. laidlawii. In press.

———, and Berg, J.: 1960. Cultivation of Mycoplasma of avian origin. Avian Dis. 4:3.

———, Fabricant, J., Yamamoto, R., and Berg, J.: 1958. Symposium on chronic respiratory diseases of poultry. I. Isolation and identification of pleuropneumonia-like organisms of avian origin. Am. Jour. Vet. Res. 19:440.

———, McMartin, D. A., and Ortmayer, H.: 1962. The effect of infectious bronchitis virus on chickens infected with Mycoplasma gallisepticum. Avian Dis. 6:267.

———, McMartin, D. A., and Shifrine, M.: 1960. Immunization against Mycoplasma infections of poultry. Am. Jour. Vet. Res. 21:482.

———, Shifrine, M., and Ortmayer, H.: 1961. Mycoplasma inocuum sp. n., a saprophyte from chickens. Jour. Bact. 82:239.

———, and Yamamoto, R.: 1956a. Preparation of a new pleuropneumonia-like organism antigen for the diagnosis of chronic respiratory disease by the agglutination test. Am. Jour. Vet. Res. 17:290.

———, and Yamamoto, R.: 1956b. Studies on chronic coryza (Nelson) in the domestic fowl. Cornell Vet. 46:337.

———, and Yamamoto, R.: 1956c. A minced chicken embryo medium for cultivating pleuropneumonia-like organisms. Poultry Sci. 35:1396.

———, and Yamamoto, R.: 1957. Pathogenic and nonpathogenic pleuropneumonia-like organisms in infectious sinusitis of turkeys. Am. Jour. Vet. Res. 18.655.

———, Yamamoto, R., and Bankowski, R. A.: 1954. A preliminary report of efficiency of various mediums for isolation of pleuropneumonia-like organisms from exudates of birds with chronic respiratory disease. Am. Jour. Vet. Res. 15:463.

———, Yamamoto, R., and Berg, J.: 1957. Strain differences of pleuropneumonia-like organisms of avian origin. Avian Dis. 1:49.

———, Yamamoto, R., and Extrom, S. F.: 1956. Control of egg-transmitted pleuropneumonia-like organisms in two hatcheries through medication of the foundation stock. Jour. Am. Vet. Med. Assn. 128:513.

Alls, A. A., Benton, W. J., Krauss, W. C., and Cover, M. S.: 1963. The mechanics of treating hatching eggs for disease prevention. Avian Dis. 7:89.

———, Cover, M. S., Benton, W. J., and Krauss, W. C.: 1964. Treatment of hatching eggs for disease prevention—factors affecting permeability and a visual detection of drug absorption. Avian Dis. 8:245.

Barber, C. W.: 1962. The lymphofollicular nodules in turkey tissues associated with Mycoplasma gallisepticum infection. Avian Dis. 6:289.

Barnes, L. E., Ose, E. E., and Ellis, L. F: 1961. Tylosin treatment of experimental *Mycoplasma gallinarum* infections of chickens and turkeys. Antimicrobial Agents Annual 1960:605.

——, Ose, E. E., and Gossett, F O... 1960. Treatment of experimental PPLO infections in young chickens with tylosin, a new antibiotic. Poultry Sci 39:1376.

Biddle, E. S., and Cover, M. S. 1957. The bacterial flora of the respiratory tract of chickens affected with chronic respiratory disease. Am. Jour. Vet. Res. 18:405.

Blake, J. T.: 1960. Effects of experimental chronic respiratory disease and infectious bronchitis on pullets. Am Jour Vet. Res. 23.847.

Brion, A., Fontaine, M., Fontaine, M. P., and Priet, C: 1958. Sur l'existence en France de la maladie respiratoire chronique des volailles. Acad. Vét. de France Bul. 31:105.

Calnek, B W., and Levine, P P. 1957. Studies on experimental egg transmission of pleuro-pneumonia-like organisms in chickens Avian Dis. 1:208.

Carson, J. R., Eaton, R. D., and Luginbuhl, R. E.: 1954. The effect of injections of an oil suspension of Terramycin on egg production and egg quality in hens affected with chronic respiratory disease. Poultry Sci 33.589

Chalquest, R., and Fabricant, J. 1959. Survival of PPLO injected into eggs previously dipped in antibiotic solutions Avian Dis. 3 257.

Chu. H. P.: 1958. Differential diagnosis and control of respiratory diseases of poultry, Vet. Record 70.1064

Chute, H. L. 1960. Pathology of PPLO and other agents in chicken embryos. Ann. N.Y. Acad. Sci 79 741

——, and Cole, C R 1954. Lesions in chicken embryos produced by pleuropneumonia-like organisms from chronic respiratory disease of chickens and infectious sinusitis of turkeys Am Jour Vet Res 15:109

Coles, J D W., and Cumming, R. B. 1959. Chronic respiratory disease: its nature and eradication. Jour. So Africsn Vet. Med. Assn. 30:5.

Cottew, G. S. 1956. A chronic respiratory disease of poultry in Queensland. Austral. Vet. Jour. 32.249

Cover, M. S. 1955 The therapeutic use of antibiotics for chronic respiratory disease in three laying flocks Poultry Sci 31 686

——, and Waller, E. F 1954. The presence of chronic respiratory disease in pipped eggs. Am. Jour. Vet. Res. 15.319.

Crawley, J. F, and Fahey, J. E 1955. A proposed plan for the control of chronic respiratory disease of chickens Poultry Sci 34 707

——, and Fahey, J. E 1957 The use of hemagglutination-inhibition test for the control of PPLO infection in poultry. Jour. Am. Vet. Med. Assn. 130.187.

de Blieck, L.: 1950 The treatment of coryza infectiosa gallinarum type II (Nelson) with streptomycin. Vet. Record 62:787.

Delaplane, J. P.: 1948. Some recent observations of lesions in chick embryos induced by the virus of a chronic respiratory disease of chickens. Cornell Vet. 38:192.

—— 1949 Cultivation of the chronic respiratory disease virus in chick embryos. Proc. 53rd Ann. Meet. U.S Livestock Sanit. Assn., p. 193.

——, and Stuart, H O 1943. The propagation of a virus in embryonated chicken eggs causing a chronic respiratory disease of chickens. Am. Jour. Vet. Res. 4.325.

Dierks, R. E: 1964. Personal communication.

Domermuth, C. H. 1958 *In vitro* resistance of avian PPLO to antibacterial agents. Avian Dis. 2.442.

——: 1960 Antibiotic resistance and mutation rates of Mycoplasma. Avian Dis. 4:456.

——: 1962. Vaccination of chickens with *Mycoplasma gallisepticum*. Avian Dis. 6.412.

——, and Johnson, R. P. 1955 An in vitro comparison of some antibacterial agents on a strain of avian pleuropneumonia-like organisms Poultry Sci. 34:1395.

Dunlop, W. R., and Strout, R. C 1956 Statewide testing for PPLO infection of poultry. Proc. 60th Ann. Meet. U.S. Livestock Sanit. Assn., p. 197.

Edward, D. G., and Kanarek, A. D.: 1960. Organisms of the pleuropneumonia group of avian origin· Their classification into species. Ann. N.Y. Acad. Sci. 79.696.

Eissa, Y. M.: 1956 La maladie respiratoire chronique des volailles en Egypte. Off. Internatl. des Epiz. Bul. 46·170

Fabricant, J.: 1953. The viability of the agent of chronic respiratory disease at minus 25° C. 25th Ann. Conference Laboratory Workers in Pullorum Dis. Control. (Abst.)

——: 1958. A reevaluation of the use of media for the isolation of pleuropneumonia-like organisms of avian origin. Avian Dis. 2:409.

——. 1959. Swine serum and CO_2 in the isolation of avian PPLO. Avian Dis. 3.428.

——: 1960. Serological studies of avian pleuropneumonia-like organisms (PPLO) with Edward's technique. Avian Dis. 4:505.

——: 1962. Personal communication.

——, and Levine, P. P.: 1962. Experimental production of complicated chronic respiratory disease infection ("air sac" disease). Avian Dis. 6 13

———, and Levine, P. P.: 1963. Infection in young chickens for the prevention of egg transmission of *Mycoplasma gallisepticum* in breeders. Proc. 17th World Vet. Cong., Hanover, Germany, p. 1469.

Fahey, J. E.: 1954. A hemagglutination inhibition test for infectious sinusitis of turkeys. Proc. Soc. Exper. Biol. and Med. 86:58.

———: 1957. Infectious sinusitis of turkeys caused by antibiotic-resistant pleuropneumonia-like organisms. Vet. Med. 52:305.

———, and Crawley, J. F.: 1954a. Studies on chronic respiratory disease of chickens. II. Isolation of a virus. Canad. Jour. Comp. Med. and Vet. Sci. 18:13.

———, and Crawley, J. F.: 1954b. Studies on chronic respiratory disease of chickens. III. Egg transmission of a pleuropneumonia-like organism. Canad. Jour. Comp. Med. and Vet. Sci. 18:67.

———, and Crawley, J. F.: 1954c. Studies on chronic respiratory disease of chickens. IV. A hemagglutination inhibition diagnostic test. Canad. Jour. Comp. Med. and Vet. Sci. 18:264.

———, and Crawley, J. F.: 1955a. Studies on chronic respiratory disease of chickens. V. Airborne spread of the CRD agents. Canad. Jour. Comp. Med. and Vet. Sci. 19.53.

———, and Crawley, J. F.: 1955b. Studies on chronic respiratory disease of chickens. VI. The effects of antibiotics on the clinical and serological course of CRD. Canad. Jour. Comp. Med. and Vet. Sci. 19.281.

———, Crawley, J. F., and Dunlop, W. R.: 1953. Studies on chronic respiratory disease of chickens. Observations on outbreaks in Canada in a two year period. Canad. Jour. Comp. Med. and Vet. Sci. 17:294.

Freundt, E. A.: 1957. Mycoplasmataies. In Breed, R. S., Murray, E. G. D., and Smith, N. R., eds., Bergey's Manual of Determinative Bacteriology. 7th ed. Baltimore, The Williams and Wilkins Co. p. 914.

Garust, A. Tello, and Nóbrega, P.: 1956. Chronic respiratory disease in Brazil. Arq. Inst. Biol., São Paulo. 23:35.

Glanforte, E. M., Junghere, E. L., and Jacobs, R. E.: 1955. A serological analysis of seven strains of pleuropneumonia-like organisms from air sac infection in poultry. Poultry Sci. 34:662.

Glantz, P. J., Narotsky, S., and Bubash, G.: 1962. *Escherichia coli* serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis. 6:322.

Gross, W. B. 1956. *Escherichia coli* as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Poultry Sci. 35:765.

———: 1961a. The development of "air sac disease." Avian Dis. 5: 431.

———: 1961b. The effect of chlortetracycline, erythromycin and nitrofurans as treatments for experimental "air sac disease." Poultry Sci. 40 885.

———: 1962. Blood cultures, blood counts and temperature records in an experimentally produced "air sac disease" and uncomplicated *Escherichia coli* infection in chickens. Poultry Sci. 41:691.

Grumbles, L. C., Phillips, E., Boney, W. A., Jr., and Delaplane, J. P.: 1953. Cultural and biochemical characteristics of the agent causing infectious sinusitis of turkeys and chronic respiratory disease of chickens. Southwestern Vet. 6:166.

Hall, C. F.: 1962. *Mycoplasma gallisepticum* antigen production. Avian Dis. 6.359.

———, Flowers, A. I., and Grumbles, L. C.: 1963. Dipping of hatching eggs for control of *Mycoplasma gallisepticum*. Avian Dis. 7:178.

———, Moore, R. W., and Grumbles, L. C.: 1963. Eradication of infectious sinusitis in a hatchery operation by serological testing. Avian Dis. 5:168.

Hamdy, A. H., Ferguson, L. C., Sanger, V. L., and Bohl, E. H.: 1957. Susceptibility of pleuropneumonia-like organisms to the action of antibiotics erythromycin, chlortetracycline, hygromycin, magnamycin, oxytetracycline and streptomycin. Poultry Sci. 36:748.

Hartwigk, H.: 1958. Nachweis von pleuropneumonia-ähnlichen organismen (PPLO) beim Geflügel. Berl. und Munch. Tierarztl. Wschr. 71:45.

Heishman, J. O., Olson, N. O., and Cunningham, C. J.: 1962. Control of chronic respiratory disease. IV. The effect of a low calcium diet and high concentrations of chlortetracycline in the isolation of Mycoplasma from experimentally infected chicks. Avian Dis. 6:165.

———, Olson, N. O., and Shelton, D. C.: 1960. Control of chronic respiratory disease. II. The effect of low calcium diet, terephthalic acid and chlortetracycline. Avian Dis. 4:413.

Hitchner, S. B.: 1949. The pathology of infectious sinusitis of turkeys. Poultry Sci. 28:106.

Hofstad, M. S.: 1952. Chronic respiratory disease. Progress Report of Vet. Med. Res. Inst., Iowa State College, Ames, Iowa. P 51.

———: 1957a. Egg transmission of infectious sinusitis in turkeys. Avian Dis. 1:165.

———: 1957b. A serological study of infectious sinusitis in turkeys. Avian Dis. 1:170.

———, and Doerr, L.: 1956. A chicken meat infusion medium enriched with avian serum for cultivation of an avian pleuropneumonia-like organism, *Mycoplasma gallinarum*. Cornell Vet. 46:439.

Hoyt, H. H., Pomeroy, B. S., and Roepke, M. H.: 1957. The propagation of the causative agent of infectious sinusitis of the turkey in the chicken embryo. Am. Jour. Vet. Res. 12:329.

Johnson, E. P.: 1954. The specificity of lymphofollicular lesions in the diagnosis of chronic respiratory disease. Cornell Vet. 44:230.

Junghcrr, E. L., Luginbuhl, R. E., and Jacobs, R. E.: 1953. Pathology and serology of air sac infection. Proc. 89th Ann. Meet. Am. Vet. Med. Assn, p. 303.

———, Luginbuhl, R. E., Tourtellotte, M., and Burr, W. E.: 1955. Significance of serological testing for chronic respiratory disease. Proc. 91st Ann. Meet. Am. Vet. Med. Assn, p. 315.

Keller, H.: 1953. Über die Isolierung von pleuropneumonie-ähnlichen Erregern bei Huhnern. Schwez. Arch. Tierheilk. 100:45.

Kelton, W. H., and Gentry, R. F.: 1957. Studies on chronic respiratory disease. II. The reversion of so-called "PPLO" to bacterial L-forms. Avian Dis. 1:347.

———, and Gentry, R. F.: 1960. Derivation of gram-positive cocci from pleuropneumonia-like organisms. Ann. N.Y. Acad. Sci. 79:410.

———, and Van Roekel, H.: 1963. Serological studies of Mycoplasma (PPLO) of avian origin. Avian Dis. 7:272

Kiser, J. S., Popken, F., and Clemente, J.: 1960. Antibiotic control of an experimental Mycoplasma gallinarum (PPLO) infection in chickens. Ann. N.Y. Acad. Sci. 79:593.

———, Popken, F., and Clemente, J.: 1961. The development of resistance to spiramycin, streptomycin and chlortetracycline by Mycoplasma gallisepticum in chick embryos. Avian Dis. 5 283.

Kleckner, A. L.: 1960. Serotypes of avian pleuropneumonia-like organisms. Am. Jour. Vet. Res. 21:274.

Lecce, J. G., and Sperling, F. G.: 1954. Chronic respiratory disease. I. The isolation of pleuropneumonia-like organisms as a diagnostic aid. Cornell Vet. 44:441.

———, and Sperling, F. G.: 1955. Chronic respiratory disease. II. The effect of treatment on the pleuropneumonia-like organism flora of avian trachea. Jour. Am. Vet. Med. Assn. 127:54.

Levine, P. P., and Fabricant, J.: 1962. Effect of dipping eggs in antibiotic solutions on PPLO transmission in chickens. Avian Dis. 6:72.

Luginbuhl, R. E., and Jungherr, E. L.: 1953. The growth curve of a chronic respiratory disease agent in embryonating eggs. Poultry Sci. 32:912 (Abst.)

McMartin, D. A., and Adler, H. E.: 1961. An immunological phenomenon in chickens following infection with Mycoplasma gallisepticum. Jour. Comp. Path. and Therap. 71:311.

Markham, F. S., and Wong, S. C.: 1952. Pleuropneumonia-like organisms in the etiology of turkey sinusitis and chronic respiratory disease of chickens. Poultry Sci. 31.902.

Mouthrop, J. M.: 1962. A report on broilers from parents free of Mycoplasma gallisepticum. Avian Dis 6:161.

Moulton, J. E., and Adler, H. E.: 1957. Pathogenesis of arthritis in chicken embryos caused by a pleuropneumonia-like organism. Am. Jour. Vet. Res. 18.731.

Nelson, J. B.: Studies on an uncomplicated coryza in the domestic fowl:
1936a. V. A coryza of slow onset. Jour. Exper. Med. 63:509.
1936b. VI. Coccobacilliform bodies in birds infected with coryza of slow onset. Jour. Exper. Med 63:515.
1936c. VII. Cultivation of the coccobacilliform bodies in fertile eggs and in tissue culture. Jour. Exper. Med. 64:749
1936d VIII. The infectivity of fetal membrane and tissue culture suspensions of the coccobacilliform bodies. Jour. Exper. Med. 64:759.
1938. IX. The cooperative action of Hemophilus gallinarum and the coccobacilliform bodies in the coryza of rapid onset and long duration. Jour. Exper. Med. 67:847.
———: 1939. Growth of the fowl coryza bodies in tissue culture and in blood agar. Jour. Exper. Med. 69:199.

Olesiuk, O. M., and Van Roekel, H.: 1952. Cultural attributes of the chronic respiratory disease agent. 24th Ann. Conference Laboratory Workers in Pullorum Dis. Control. (Abst.)

———, and Van Roekel, H.: 1959. The effects of antibiotics on experimental chronic respiratory disease in chickens. Avian Dis. 3:457.

———, Van Roekel, H., and Bennato, L. P.: 1957. Influence of chemotherapeutic agents on experimental chronic respiratory disease in chickens and turkeys. Poultry Sci. 36:383.

———, Van Roekel, H., and Chandramani, N. K.: 1961. Antibiotic medication of chickens experimentally infected with Mycoplasma gallisepticum and Escherichia coli. Avian Dis. 8:135.

Olson, N. O., Hash, T. R., Heishman, J. O., and Campbell, A.: 1962. Dipping of hatching eggs in erythromycin for the control of Mycoplasma. Avian Dis. 6:191.

———, Heishman, J. O., and Cunningham, C. J.: 1964. Control of chronic respiratory disease. VI. The effect on egg transmission of early exposure of chicks to Mycoplasma gallisepticum. Avian Dis. 8:215.

———, Heishman, J. O., and Shelton, D. C.: 1960. Control of chronic respiratory disease. III. Isolation of Mycoplasma as influenced by potentiated chlortetracycline and time interval after exposure. Avian Dis. 4:419.

———, Heishman, J. O., and Shelton, D. C.: 1962. Control of chronic respiratory disease. V. Artificial exposure of young chicks to Mycoplasma gallisepticum. Avian Dis. 6:171.

——, Shelton, D. C., and Heishman, J. O.: 1959. Control of chronic respiratory disease. I. The effects of chlortetracycline (CTC) and terephthalic acid (TPA). Avian Dis. 3:443.

Osborn, O. H., and Pomeroy, B. S.: 1958. Case report—isolation of the agent of infectious sinusitis of turkeys from naturally infected pheasants. Avian Dis. 2:370.

Pathak, R. C., and Singh, C. M.: 1963. Occurrence of pleuropneumonia-like organisms in poultry in India. Agra Univ. Jour. of Res. Sci. 10(II):155.

Peterson, J. H.: 1953. Terramycin injections control chronic respiratory disease in two pullet flocks. Vet. Med. 48:311.

Prier, J. E., and Dart, G.: 1951. Immunological and serological studies on infectious sinusitis of turkeys. Cornell Vet. 41:38.

Quizon, A. S.: 1958. A survey of the occurrence of chronic respiratory disease (CRD) in the Philippines. Philippine Jour. Anim. Indu. 19:69.

Reagan, R. L., Day, W. C., and Brueckner, A. L.: 1953. Electron microscopy studies of four strains of chronic respiratory disease agent. Poultry Sci. 32:960.

Shifrine, M., Pangborn, J., and Adler, H. E.: 1962. Colonial growth of Mycoplasma gallisepticum observed with the electron microscope. Jour. Bact. 83:187.

Smith, W. E., Hillier, J., and Mudd, S.: 1948. Electron micrograph studies of two strains of pleuropneumonia-like (L) organisms of human derivation. Jour. Bact. 56:589.

Somerson, N. L., and Morton, H. E.: 1953. Reduction of tetrazolium salts by pleuropneumonia-like organisms. Jour. Bact. 65:245.

Stricker, F., and Fišera, J.: 1955. Chronická chroba dýchodiel hydiny. Veterinárský Časopis 6:199.

Stuart, E. E., and Bruins, H. W.: 1963. Preincubation immersion of eggs in erythromycin to control chronic respiratory disease. Avian Dis. 7:267.

Tajima, M., Miyamoto, T., and Nagashima, M.: 1958. An outbreak of chronic respiratory disease of chickens in Japan. Nippon Institute for Biological Science Bulletin of Biological Research. Tachikawa, Tokyo, Japan. 3:43.

Taylor, J. R. E., and Fabricant, J.: 1957. Studies on the isolation of the pleuropneumonia-like organism of chronic respiratory disease of fowls. Cornell Vet. 47:112.

——, Fabricant, J., and Levine, P. P.: 1957. A comparison of four in vitro methods for the isolation of the pleuropneumonia-like organism of chronic respiratory disease from tracheal exudate. Avian Dis. 1:101.

Thompson, C. H., Jr.: 1954. Propagation of a mixed culture of a chronic respiratory disease agent and Newcastle disease virus in chicken embryos. Am. Jour. Vet. Res. 15:295.

Van Roekel, H., Gray, J. E., Shipkowitz, N. L., Clarke, M. K., and Luchini, R. W.: 1957. Etiology and pathology of the chronic respiratory disease complex in chickens. Bull. 486 Univ. Mass., Amherst, Mass.

——, and Olesiuk, O. M.: 1953. The etiology of chronic respiratory disease. Proc. Am. Vet. Med. Assn., 1953, p. 289.

——, Olesiuk, O. M., and Beninato, L. P.: 1958. Symposium on chronic respiratory diseases of poultry. III. Epizootiology of chronic respiratory disease in chickens. Am. Jour. Vet. Res. 19:453.

——, Olesiuk, O. M., and Peck, H. A.: 1952. Chronic respiratory disease of chickens. Am. Jour. Vet. Res. 13:252.

Wasserman, B., Yates, V. J., and Fry, D. E.: 1954. On so-called air-sac infection. Poultry Sci. 33:622.

White, F. H., Wallace, G. I., and Alberts, J. O.: 1954. Serological and electron microscope studies of chronic respiratory disease agent of chickens and of turkey sinusitis agent. Poultry Sci. 33:500.

White-Stevens, R., and Zeibel, H. G.: 1954. The effect of chlortetracycline (Aureomycin) on the growth efficiency of broilers in the presence of chronic respiratory disease. Poultry Sci. 33:1164.

Wichmann, R. W.: 1957. Case report—PPLO infection in chukar partridges (Alectoris graeca). Avian Dis. 1:222.

Wills, F. K.: 1955. Isolation of pleuropneumonia-like organisms from a peacock (Pavo cristatus). Southwestern Vet 8:258.

Winterfield, R. W.: 1955. Pigeons as a source of the turkey sinusitis agent. Vet. Med. 48:124.

Wong, S. C., and James, C. G.: 1953. The susceptibility of the agents of chronic respiratory disease of chickens and infectious sinusitis of turkeys to various antibiotics. Poultry Sci. 32:589.

Yamamoto, R., and Adler, H. E.: 1956. The effect of certain antibiotics and chemical agents on pleuropneumonia-like organisms of avian origin. Am. Jour. Vet. Res. 17:538.

——, and Adler, H. E.: 1958a. Characterization of pleuropneumonia-like organisms of avian origin. I. Antigenic analysis of seven strains and their comparative pathogenicity for birds. Jour. Inf. Dis. 102:143.

Yamamoto, R., and Adler, H. E.: 1958b. Characterization of pleuropneumonia-like organisms of avian origin. 11. Cultural, biochemical, morphological and further serological studies. Jour Inf. Dis. 102:243.

Yoder, H. W., Jr., and Hofstad, M. S.: 1962. A previously unreported serotype of avian Mycoplasma. Avian Dis. 6:147.

———, and Hofstad, M. S.: 1964a. Characterization of avian Mycoplasma. Avian Dis. 8:481.

———, and Hofstad, M. S.: 1964b. Evaluation of tylosin in preventing egg transmission of Mycoplasma gallisepticum in chickens. Manuscript submitted to Avian Dis.

———, Nelson, C. L., and Hofstad, M. S.: 1961. Tylosin, an effective antibiotic for treatment of PPLO turkey sinusitis. Vet. Med. 56.178

Zander, D. V.: 1961. Origin of S6 strain Mycoplasma. Avian Dis. 5:154.

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14

Brucellosis, Anthrax, Pseudotuberculosis, Tetanus, Vibrio Infection, Avian Vibrionic Hepatitis, and Spirochetosis*

Brucellosis

Brucellosis is the name applied to the disease caused by the three species of the genus *Brucella*. In the initial stages of the infection there may be a bacteremia. After the bacteria recede from the blood stream, the disease continues in a subacute or chronic form with or without outward manifestations.

Occurrence. This disease is common in most countries in cattle, goats, swine, and man, and it also affects other mammals. It does not seem to be prevalent in birds. Veterinary literature contains several reports dealing with natural and experimental infection in various avian species. However, most of the diagnoses of brucellosis in fowl have been based on the agglutination test or merely on the fact

that the sick birds were or had been in contact with infected mammals. Very rarely have any of the Brucella been isolated from naturally infected birds. Therefore, the possibility exists that the outbreaks of disease diagnosed as brucellosis might have been caused by something else. Positive agglutination tests cannot be accepted as proof of Brucella infection. Such reactions might be due to continued ingestion of material contaminated with avirulent Brucella or they may have been nonspecific (Shklair and Stafseth, 1954).

History. According to several authors, brucellosis was first observed by Fiorentini in Italy in 1906. His diagnosis was based on the fact that 55 per cent of the birds reacted positively to the *Br. abortus* agglutination tests. Dubois (1910) reported on an outbreak of a disease in chickens, with a 75 per cent mortality, which he regarded as brucellosis because the birds were in contact with sheep suffering from *Br. melitensis* infection, and 60 per cent

[427]

of the affected birds reacted to the melitensis agglutination test in dilutions of 1.50 to 1:600. Zwick and Zeller (1913) unsuccessfully attempted to infect fowl by repeated subcutaneous, intramuscular, intraperitoneal, and intravenous injections of *Br. abortus.* Koegel (1923) tried to produce infection in chickens and pigeons by feeding and injecting large doses of *Br. abortus.* These birds gave agglutination reactions in titers of 1·200 to 1 1000 but showed no symptoms Emmel and Huddleson (1929) and Huddleson and Emmel (1929) claimed to have produced infection in fowl by feeding naturally infected milk, portions of an aborted fetus, and cultures They also claimed to have found natural Brucella infection in four flocks. A year later Emmel (1930b) found that turkeys, pheasants, ducks, and geese could be infected by feeding massive doses of the organism During the same year he found 16.5 per cent reactors to the agglutination test in a flock of 90 chickens (Emmel, 1930a). Many of these birds were in poor condition, and the egg production was low. According to Anguelov (1931) brucellosis is very prevalent, especially in modern poultry plants in Bulgaria. McNutt and Purwin (1930a, 1930b) tested 20 flocks with the agglutination test and found only a small percentage of reactors, none of them showing symptoms Attempts at producing infection by feeding and injection failed, as no symptoms were shown and no deaths occurred They further tested 10,000 birds in 69 flocks and obtained less than 2 per cent reactors, the highest percentage in any one flock being 12. Here again no evidence of illness was observed Strange and Beach (1931) failed to produce clinical disease in 32 chickens by feeding and injection of cultures. Gilman and Brunett (1930) tested 4 flocks of chickens, finding only a small percentage of reactors to the agglutination test. They conclude, however, that the evidence points to the presence of natural infection in farm flocks. McNutt and Purwin (1932) found laying pullets susceptible to infection with Brucella to the extent that egg production

was slightly decreased temporarily. In 10-day-old chicks no ill effects were produced. Van Roekel and his co-workers (1932) tested 25,202 chickens in 53 flocks with the agglutination test. The area covered represented approximately every county in Massachusetts. The total chicken population in these flocks was 70,479 birds. Two dilutions (1:25 and 1:50) were used. No reactors were found. Thirty flock owners had other livestock on the premises, and nine permitted the chickens to come in contact with the livestock. On three farms where reactors were found among cattle, the chickens were not allowed to come in contact with the cattle.

Two hens were given twelve feedings of a saline suspension of the organism during a period of 17 days, and agglutinins were detected 16 days after the first feeding. However, at no time were agglutinins well established in the blood stream, and the titer began to decrease two weeks after agglutinins were first detected. The birds were killed 28 days after the first feeding. Pathological and bacteriological findings were negative. Two hens were given three intraperitoneal inoculations with a saline suspension of the organism. Agglutinins were detected 7 days after the first inoculation, and a strong titer had been established at 4 weeks. At the end of 7 weeks the birds were killed, and no pathological or bacteriological evidence of infection was observed. Two males were given nine intraperitoneal inoculations with a saline suspension of the organism. Inappetence, somnolence, ruffled feathers, and decrease in body weight resulted. Agglutinins were present in the blood stream 9 days after the first feeding. Necropsies were performed 20 days after the first feeding, and *Br. abortus* was recovered from both individuals. One male was given three intravenous inoculations with a saline suspension of the organism. Clinical symptoms were observed. Agglutinins were established in the blood stream between the fourth and nineteenth days after the first inoculation. A necropsy was performed on the nineteenth day and *Br.*

abortus was recovered. These observations show that natural Brucella infection in chickens in Massachusetts appears to be of little, if any, significance. Agglutinins were produced when birds were fed and inoculated with saline suspensions of the organism. Repeated doses of the antigen were tolerated.

Beller and Stockmayer (1933) showed that chickens can be infected via natural and artificial channels. They found that the agglutination titer may be high 1½ years after infection. The organisms disappeared rapidly from the blood and organs and were not transmitted to the eggs. Young chickens were relatively resistant. Chickens injected with the organisms developed agglutination titers as high as 1:10,000. The organisms were re-isolated from twelve of thirty-two chickens. Five of these were injected intravenously, six intramuscularly, and one intraperitoneally. In most cases the organisms were obtained from the spleen, liver, and bone marrow. Thomsen (1934) exposed 2,677 chickens to natural infection. Only 15 per cent of his birds developed agglutination titers of 1:50. He concluded that brucellosis in chickens is of no importance in Denmark. Liddo (1934) exposed birds to infection with *Br. melitensis* of human origin and found pigeons more susceptible than chickens. Pavlov (1938) reported on his experiments with brucellosis in birds. He found that birds are less easily infected than mammals by natural and artificial methods, and that chickens are more susceptible than pigeons. Five of seven rabbits placed with infected chickens became infected and died within three months. Pure cultures of Brucella were isolated from them. None of three normal chickens and ten normal guinea pigs placed with infected chickens developed any evidence of infection. Eggs from chickens injected with massive doses of Brucella were found to contain the organisms only between the fourth and the fourteenth day after the injection. None of the injected birds developed any symptoms. The allergic and the agglutination tests were

found to be effective procedures in the detection of infection. The former gives the most pronounced reaction 24 hours after the injection of the test substance and is applicable three months after infection. The agglutination titer reached its maximum one month after infection. Two months after infection the agglutinins disappeared from the blood. Brucella injected in small doses (0.01 cc.) into eggs kept at ordinary temperature retained their virulence for one month and perhaps longer. Pagnini (1939) attempted to infect chickens by giving them gelatin capsules containing Brucella. The purpose of his work was to determine the role of chickens in the spread of brucellosis. Since he succeeded only when employing huge quantities of organisms, he concluded that chickens are of no practical significance in the spread of this disease. Felsenfeld *et al.* (1951) studied artificial Brucella infection in young chickens and found that intramuscular and intraperitoneal injection as well as feeding with Brucella caused bacteremia, fecal excretion of organisms, and significant agglutinin response. Cross reactions with *Vibrio cholerae, Proteus OX19,* and *Salmonella pullorum* were observed. Pen contact infection also occurred.

Anczykowski (1954) tested 29 flocks of chickens (2,384 individuals) with the agglutination test for brucellosis in Lower Silesia. Of these, 25 flocks (86.2 per cent) reacted positively in a 1:25 dilution or higher. The percentage of reactors in the various flocks varied from 1 to 55.5. Of 46 turkeys tested, 41.3 per cent reacted. Milk from infected cows or dairies was thought to be the source of infection. No correlation between positive and negative Brucella and *S. pullorum* agglutination reactions was established. The results, as a whole, suggest that the tests are specific.

Galuso and Rementzova (1955), in a review of research by Russian workers on brucellosis with special reference to this disease in wild animals, question the validity of the contention that goats and sheep are the original reservoirs of brucel-

losis They believe that the disease exists in wildlife including mammals, birds, reptiles, amphibians, and even fish. The role of ticks and mites in the spread of brucellosis is discussed.

Jurado et al (1951) studied experimental brucella infection in chickens and found that agglutinins were produced There were no signs or lesions of disease and the bacteriological findings were negative. They concluded that chickens in Brazil are scarcely, or not at all, significant as a reservoir of *Brucella abortus* and consequently of no epidemiological importance.

Spread. Felsenfeld *et al.* (1951) demonstrated transmission from artificially infected chicks to uninfected pen mates. The organism was found in droppings from infected birds. They also demonstrated that other diseases, such as coccidiosis or pullorum disease, increased the susceptibility of chickens to brucella infection. It appears that avian species become infected only under conditions of severe exposure or under other circumstances which decrease normal resistance. Spink (1956) noted the marked resistance of chickens to *Brucella abortus* and *Brucella melitensis* in contrast to the marked susceptibility of the chicken embryo. The conclusion was that "it is doubtful that chickens or other birds can be considered a significant reservoir of brucellosis." This conclusion is in agreement with that of Pagnini (1939).

Symptoms. Most authors have failed to observe symptoms of brucellosis in birds, and it is not certain that the symptoms described by others have actually been due to this disease. Dubois (1910) attributed the following symptoms to brucellosis. Between the months of March and June, 1910, 140 of 200 birds died. Young as well as old birds were affected. The disease appeared in a peracute and subacute form, killing birds in a few hours to 8 to 10 days. In the peracute form the birds died without previous symptoms. In the subacute form there was first loss of appetite; then the birds became weak and walked with difficulty. During the last 3 to 4 days they squatted and crowded together with ruffled feathers and drooping wings; they could easily be caught; sometimes they had greenish diarrhea. At last they became extremely emaciated. The following symptoms were recorded by Emmel and Huddleson (1929): "The birds first went off egg production and usually developed severe diarrhea. A gradually increasing paleness about the head, comb, and wattles, and emaciation occurred. Before death the birds became weak and often showed paralysis. The course of the disease ranged from 18 to 46 days."

Pathology. Dubois (1910) observed swelling of the spleen and liver and petechiae on the lungs. Emmel and Huddleson (1929) recorded the following anatomical changes: In the early stages of the disease the spleen was generally enlarged; later in the course of the disease it became shrunken. The liver was pale, mottled, and showed numerous brownish or gray foci on the surface; at death it had become pale and friable. The kidneys were also pale and showed some degeneration. The ovary was in the process of atrophy with flaccid ova of a dirty yellowish color. The intestines showed inflammation with some necrosis. In the duodenum there were irregular, elevated areas of cell infiltration. Beller and Stockmayer (1933) mention enlargement of the liver and very rare occurrence of necrotic foci in the spleen.

Diagnosis. The isolation and identification of one of the members of the genus Brucella constitute the only positive diagnosis. To be sure, agglutination and allergic tests have been employed successfully in experimental work. However, positive tests may be obtained with birds that cannot be proved to be infected by bacteriological examination, and Emmel and Huddleson (1929) found that the birds often gave a negative agglutination test in the last stages of the disease. Pavlov (1938) found that the agglutinins disappeared from the blood two months after infection, while Beller and Stockmayer (1933) found agglutinins in the blood a year and a half after infection. The difference between the

observations of Pavlov and Beller and Stockmayer may be due to a difference in the duration of the infection. According to Pavlov the allergic state remains longer than do the agglutinins in the blood. The allergic test was made by injecting a test substance, prepared according to Dubois (1933), into the skin at the point of the wing after removal of a few feathers.

Treatment and prevention. A number of drugs including the tetracyclines, chloramphenicol, the streptomycins, sulfadiazine, and corticosteroids have been found to be effective for treating brucellosis in man. They have not been tested in poultry. Although similar beneficial effect may be anticipated in treating poultry, it is doubtful if treatment would be indicated except under unusual circumstances. Since

birds can become infected with Brucella and may thus serve as agents of transmission, not only to other birds but to mammals as well, one should take steps to prevent fowl from being in contact with infected mammals. Good poultry hygiene demands that poultry should be confined within premises set aside for this type of livestock and not be allowed in barns, hog yards, etc. The practice of throwing dead chickens on the manure pile or elsewhere, where hogs or other birds may eat them, is to be condemned. Should a farm flock be found to be affected with brucellosis, the safest thing to do is to destroy the flock, disinfect the premises, and restock after leaving the poultry house and yards idle for several months. The testing and slaughter method is not applicable in poultry practice.

REFERENCES

Anczykowski, F.: 1954. Brucellosis in fowls. II. Serology. III. Agglutination tests with serum of fowls with concurrent S. pullorum and Br. abortus infection. Roczn. Nauk rol. Ser. E 66:303 and 319.

Anguelov, S.: 1951. Bul. de l'Office Internat. des Epizooties.

Beller, K., and Stockmayer, W.: 1935. Die Pathogenität der Br. abortus für Hühner und Kücken. Deutsch. tierärztl. Wochenschr. 41:551.

Dubois, G.: 1933. Dépistage des Brucella chez la poule par la cherche des réactions d'allergies Compt. rend. Soc. de biol. 115:1045.

Dubois, M.: 1910. Malta fever in fowls. Rev. Vet. 67:490.

Emmel, M. W.: 1950a. An outbreak of Brucella disease in the fowl. Jour. Am. Vet. Med. Assn 76 564.

————. 1950b. The susceptibility of the turkey, pigeon, pheasant, duck, and goose to Brucella disease. Jour. Am. Vet. Med. Assn. 77.185.

————, and Huddleson, I. F.: 1929. Abortion disease in the fowl. Jour. Am. Vet. Med 77:578.

Felsenfeld, O., Young, V. M., Loeffler, E., Ishihara, S. J., and Schroeder, W. F.: 1951. A study of the nature of Brucellosis in chickens. Am. Jour. Vet. Res. 12:48.

Galuso, I. G., and Rementsova, M. M.: 1955. Reservoir of animal brucellosis among wild animals and birds in the light of studies of foci of infection. Trud. Inst. Zool. Alma-Ata, 3:12.

Gilman, H. L., and Brunett, E. L.: 1930. Bact. abortus infection in the fowl. Cornell Vet. 20:371.

Huddleson, I. F., and Emmel, M. W.: 1929. The pathogenicity of the species of the genus Brucella for the fowl. Mich. Agr. Exper. Sta., Tech. Bul. 105

Jurado, F. R., Mazzini, G. A., Cedro, V. C. F., and Torre, E. J.: 1951. Experimental brucellosis in fowls. Proc. 5th Vet. Cong. Bras. Vet. São Paolo, 1950, p. 629.

Koegel, A.: 1925. Beiträge zur Abortusforschong. Munchener tierärztl. Wochenschr. 74:617.

Liddo, S.: 1934. Brucellosi aviaria sperimentale. Bol. Accad. Pugliese Sci., p. 100.

McNutt, S. H., and Purwin, P.: 1930a. The effect of the Brucella group of microorganisms on chickens. Jour. Am. Vet. Med. Assn. 77:212.

————, and Purwin, P.: 1930b. The effect of the Brucella group of microorganisms on chickens. Jour. Am. Vet. Med. Assn. 77.350

————, and Purwin, P.: 1932. Feeding of Brucella organisms to chickens and its effect on egg production of pullets and on growth of young chicks. Jour. Am. Vet. Med. Assn. 81.641.

Pagnini, Ugo: 1959. I polli nella diffusione delle brucellosi. La Clin. Vet. 62:75.

Pavlov, P.: 1938. La brucellose chez les volailles. Rec. de Méd. Vét. 114:790.

Shklair, I. L., and Stafseth, H. J.: 1954. A study of serological cross reactions between the Brucella and certain salmonellae. Mich. St. Coll. Veterinarian 14-130.

Spink, W. W.: 1956. Brucellosis. The Lund Press, Inc., Minneapolis, p. 78.

Strange, C. R., and Beach, B. A.: 1931. Are chickens susceptible to contagious abortion. Vet. Med. 26:4

Thomsen, A.: 1931. Sur la présence de l'infection à Brucella dans les effectifs de Volaille du Danemark. Bul de l'Office Internal. des Epizooties. 7:1057.

Van Roekel, H., Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1932. Susceptibility of chickens to brucellosis. Jour. Am. Vet. Med. Assn. 80.641.

Zwick and Zeller: 1913 Über den infektiösen Abortus des Rindes. Arb. a. d. kaiserl. Gesundheitsamt 43 1.

Anthrax

Anthrax is caused by *Bacillus anthracis* which in susceptible hosts produces an acute septicemic (bacteremic) infection characterized mainly by fever, slight coagulation and dark discoloration of the blood, swelling of the spleen, and edema and hemorrhages in various tissues.

Anthrax occurs rarely in birds. The rather frequent reports on cases of avian anthrax found in the literature of the nineteenth century were not based on bacteriological examinations, and it seems reasonable to assume that the great losses recorded may have been due to cholera or plague. The opinion expressed by Pasteur (1878), that chickens under ordinary conditions are resistant to anthrax, is shared by nearly all authors. Heusinger (1850) claimed that fowl could be fatally infected with anthrax. He is also responsible for the supposition that this disease occurred in epidemic form among chickens in Germany during the years 1832 and 1835.

EXPERIMENTAL WORK WITH AVIAN ANTHRAX

Artificial infection. Feser (1879) injected twenty-four chickens subcutaneously, and gave others anthrax bacilli in their feed for weeks without producing a single case of anthrax. Oemler (1879) fed anthrax material to eight ducks and twenty-eight chickens. The ducks sickened in 24 hours and soon died, while none of the chickens became ill. Only negative results were obtained by Koch et al. (1884) who gave their chickens large numbers of spores in feed. Perroncito (1885), Kitt (1886), and Hess (1887) were unsuccessful in their attempts to produce anthrax in chickens. In extensive experiments with anthrax in chickens, Hofherr (1910) failed to produce the dis-

ease even when giving large quantities of anthrax material to birds that were greatly weakened by starvation, thirst, cold baths, feeding of powdered glass, lime, vegetable diet, etc. By feeding a very large quantity of infectious material, one rooster that was already sick and one chick were infected. Fatal infection was produced, by feeding, in 25 per cent of the ducks and 100 per cent of the pigeons employed in his experiments. Thus, Hofherr showed that pigeons and ducks are susceptible to anthrax and that healthy chickens are ordinarily resistant but not always completely immune. Hunger and youth predispose to infection and so does lowered body temperature as was shown by Pasteur in 1878.

Mechanism of resistance. The mechanism of resistance of chickens to anthrax infection was studied very carefully by Wagner (1890). His work on forty-six chickens showed that under ordinary conditions chickens are resistant to this disease and that the immunity is due to phagocytic action of leukocytes. Anthrax bacilli can grow and maintain their virulence in the body of the chicken. The presence of the organism in the tissues is not without effect as shown by fever and cellular infiltration at the point of local infection. The resistance of chickens can be broken down by placing them in cold water baths for several hours or by giving them antipyrin or chloral hydrate. Submerging the chickens in a water bath for several hours resulted in death from anthrax in all of six birds. Antipyrin lowered the resistance to the extent that six out of eleven birds died of anthrax. In the case of chloral hydrate only one bird out of eight died. Lowering of the body temperature was shown to interfere with the mi-

gration of leukocytes to the point of infection. By use of the cold water bath the body temperature could be brought to a lower level and held there longer than by antipyrin and chloral hydrate. This may be the reason why the cold water bath reduced resistance more effectively than the other two agents. Möllhoff (1910) tried to determine the reason for the great resistance of birds to anthrax, and, on the basis of his experiments, drew the conclusion that the bactericidal action of lymph and blood play an important part, and that the high body temperature of the birds is less important.

NATURAL INFECTION

Anthrax in chickens. The only definitive diagnosis of anthrax reported in chickens was made by Serafimov (1961). The organism was isolated from the bone marrow of two dead hens. Lesions were not described. Oral administration of massive doses of the isolates to normal chickens failed to produce the disease. The hens had been in a yard in which a heifer had died of anthrax within the month. It was speculated that nutritional deficiencies existing in the birds at the time of infection, coupled with massive exposure, contributed to the infection. The nutritional deficiencies were not specified.

Anthrax in the ostrich. Epidemics of anthrax have been observed in the ostrich. The disease usually runs an acute course, but subacute cases occur, often terminating in recovery. Anthrax in the ostrich was first reported by Henning in 1894. Robertson (1908) reported on a case in an ostrich, and later Theiler (1912) and Ward and Gallagher (1920) described the disease in detail.

Pathology. Anthrax bacilli are present in all organs and do not differ, even with respect to pathogenicity, from those isolated from mammals. The following tissue changes may be observed: Slight coagulation and dark discoloration of the blood, increase in the fluid in the thoracic and abdominal cavities; petechial and larger hemorrhages in the pericardium, peritoneum, and mesentery; sometimes gelatinous infiltrations in the subcutaneous and deeper tissues; very frequently there is hemorrhagic enteritis; edematous swelling and hemorrhages in many places in the submucosa; not infrequently there may be a mass of blood in the lumen of the colon; the spleen, liver, and kidneys show swelling and congestion with blood; as a rule the lungs and stomach appear normal.

Anthrax in ducks. In birds other than the ostrich, anthrax appears only sporadically. Gerlach (1923) described a case of natural infection in a duck. This case occurred shortly after a few pigs had died of anthrax on the same farm. Ubertini (1939) reported on an outbreak of anthrax in ducks. These ducks were kept with about 100 chickens. There were two varieties of ducks — the common kind and five "mute" ducks (Cairina moschata) These five became ill and died while none of the others nor the chickens were affected. This outbreak occurred about 10 days following the death of a cow from anthrax.

Pathology. The following tissue changes have been observed: edematous swelling of the head, throat, and upper part of the neck, sometimes extending along the entire length of the esophagus, resembling a sac filled with fluid; the skin bluish-red in the affected areas; cyanosis of the mucosa of the head; gelatinous infiltration of the pharyngeal mucosa; inflammation of the intestinal mucosa; and other changes such as those recorded in the following description by Almeyew of a case of anthrax in an eagle.

Anthrax in birds in zoological gardens. Almeyew (1936) reported that anthrax had been observed in zoological gardens following the feeding of meat containing large numbers of anthrax bacilli. The source of the meat was unknown. He gave detailed results of a necropsy on the carcass of an eagle that had died of anthrax. This eagle was sent from the Zoological Garden in Kasan, eastern Russia, where it had died suddenly May 12, 1935.

The postmortem findings were mainly as follows: The nutritional state of the

carcass was fair; there was only slight muscular rigidity. The visible mucous membranes of the oral cavity showed a pale gray color and thick slimy coating, on the removal of which one could observe passive hyperemia. The tongue was covered with a slimy mass. The spleen was greatly enlarged; the capsule was distended and the pulp soft, discolored, and moist; in the pulp there were sharply circumscribed nodules from the size of pinheads to that of millet seed, reddish-brown or grayish-red in color; the cut surface was dull. The liver was enlarged, distended, and hyperemic; under the capsule and throughout the parenchyma there were hemorrhagic and necrotic foci. The cut surface was moist, and the vessels were distended. The kidneys were much enlarged, hyperemic, spotted, gray-yellowish-brown in color and were studded with hemorrhages and necrotic foci. The cut surface was moist, and the exuding blood was only slightly coagulated; the cortical and medullary layers were hyperemic and studded with grayish-red foci. The adrenals were edematous, hyperemic, and showed numerous hemorrhages; the serous membrane was dull and showed petechial hemorrhages. The lungs were edematous, hyperemic, and dark bluish-red in color; the surface was moist and studded with petechial hemorrhages. The pericardium was distended, of dull bluish-red color, and contained a yellowish-red transudate. The heart muscle was soft, being pale loam-colored and parboiled in appearance. The stomach was collapsed and grayish-blue; the vessels of the serosa were filled with blood; the stomach wall was thickened, the mucous membrane wrinkled, dark, and covered with mucus. The duodenum was dark red and showed numerous petechial hemorrhages, and the mucous membrane was covered with a reddish-brown mucous mass. The surface of

the large intestine was dark brownish-red, the walls thickened, and the mucous membrane covered with a brownish mucous layer.

Mollet (1913) and Urbian (1946) point out the possibility of mechanical dissemination of *B. anthracis* by free flying birds feeding on infected cadavers and offal.

Diagnosis. A presumptive diagnosis of anthrax in birds may be established by microscopic examination of stained films from blood, edematous fluid, or tissues in which anthrax bacilli usually are present in large numbers. It is well to confirm the diagnosis by cultural methods because of the possibility of mistaking saprophytic organisms for anthrax bacilli. The Ascoli precipitation test is a quick and reliable diagnostic procedure.

Treatment and prevention. The prophylactic value of vaccination of birds is unknown; the rare occurrence of the disease probably would not warrant its use even if effective. Specific antiserum as well as penicillin and oxytetracycline have proved effective in treating the disease in mammals. The value of these treatments for the disease in birds is unknown. Sanitary methods should be used to prevent spread of infection. Sick birds should be removed promptly, killed, and burned. Under no circumstances should dead birds be allowed to lie around so that other birds may pick at them or eat them. Contaminated ground should be fenced off, and houses should be thoroughly cleaned and disinfected. Litter, rubbish, and equipment of little or no value should be burned. Valuable birds that show no symptoms may be confined, preferably in houses with good cement floors where proper disinfection is possible. If the birds are few in number and of little value, it may be preferable to destroy the whole flock to facilitate the cleaning-up process.

REFERENCES

Almejew, H. S.: 1956 Anthrax beim Vogel. Deutsch. tierärztl. Wochenschr. 44:375.
Feser: 1879. Über Infektionsversuche mit Milzbrand beim Hausgeflügel. Adams Wochenschr. [Quotation from Kitt (p. 89)]
Gerlach, F.: 1923. Bemerkenswerter Verlauf einer Milzbrandenzootie. Wiener tierärztl. Monatschr. 10 481.

Hess, C.: 1887. Untersuchungen zur Phagocytenlehre. Virchow's Archiv. 109:365.

Heusinger, C. F.: 1850. Milzbrand Krankheiten der Thiere und des Menschen. Enke, Erlangen.

Hofferr, O.: 1910. Experimentelle Beiträge zur Milzbrandinfektion des Geflugels durch Futterung. Zentralbl. f. bakt. I. Orig 55:434.

Kitt, T.: 1886. Kleinere Mitteilungen aus der pathologischen Abtheilung und Seuchenversuchsstation. Jahresbr. der K. Central-Thierarzneischule in Munchen (1884–85). Suppl. Deutsch. Zeitschr. f. Thiermed. u. vergleich. Path. 12:85.

Koch, R., Gaffky, G., and Loeffler, F. A. J.: 1884. Experimentelle Studien uber die künstliche Abschwachung der Milzbrandbacillen und Milzbrandinfektion durch Fütterung. Mittheilung des Gesundheitsamt. 2:174.

Mollet, F.: 1913. Die Bedeutung von Krahe und Fuchs fur die Verbreitung des Milzbrandes. Zentralbl. Bakt. 70:19.

Mollhoff: 1910. Untersuchungen uber die Empfanglichkeit des Geflugels fur Milzbrand und uber die Grunde der Resistenz des Huhnes gegen diese Krankheit. inaug. Dissert., Univ. Bern.

Oemler, H.: 1879. Experimentelle Beitrage zur Milzbrandfrage. Arch. f. wiss. u. prakt. Thierheilk. 5:164.

Pasteur, L.: 1878. Charbon et virulence. Bul. de l'Acad. de Med. 43:253.

Perroncito, E.: 1885. Carbonchio nei polli, p. 159: it Carbonchio, Mezzi preventivi e curativi. Torino. [Quotation from Kitt (p. 90).]

Robertson, W.: 1908. Case of anthrax in an ostrich. Jour. Comp. Path. and Therap. 21:361.

Sarafimov, S.: 1961. A case of anthrax in poultry. Parariml Bolesti. Irv. 1:217.

Theiler, A.: 1912. Anthrax in the ostrich. Agr. Jour. of Union of So. Africa 4:370.

Ubertini, B.: 1939. Un focolaio di inferione carbonchiosa ad insorgenza spontanea nelle anitre (Cairina Moschata). La Clinica Vétérinaria 62:72.

Urban, A.: 1946. Possibilité de dispersian des bacilles tuberculeux et de spores charboneuses par de déjections d'oiseaux carnivores. Bul. Acad. Vét. Fr. 19:237.

Wagner, R. E.: 1890. Contribution à l'étude de l'immunité. Le Charbon des Poules. Ann. de l'Inst. Past. 4:370.

Ward, A. R., and Gallagher, B. A.: 1920. Diseases of Domesticated Birds. The Macmillan Co., New York.

Pseudotuberculosis

Pseudotuberculosis is a contagious disease caused by *Pasteurella pseudotuberculosis*, usually characterized by an acute septicemia of short duration, followed by a chronic focalized infection which gives rise to tubercular lesions in various organs.

History. The first isolation of the bacillus of pseudotuberculosis was made from a subcutaneous tubercular lesion on the forearm of a child by Malassez and Vignal (1883). Malassez published another report on this organism in 1884. Rieck (1889) reported on an outbreak of a disease in canaries which, according to the description of the symptoms, lesions, and characteristics of the isolated organism, must have been pseudotuberculosis. Several other authors have reported on this disease in canaries: Zürn (1884), v. Wasielewski and Hoffman (1903), Pfaff (1905), Freese (1907), Miessner and Schern (1908), Zwick (1914), Zeiss (1914), and van Heelsbergen (1927).

Woronoff and Sineff (1897) reported that they had found a case of pseudotuberculosis in a chicken. Truche and Isnard (1937) reported on an outbreak of this disease in a flock of adult chickens, and Schäfer (1939) reported on two cases in 14-day-old Leghorn chicks.

Bryner (1906) reported on an outbreak that occurred in 1904 involving tiger finches (*Habropya amandara* 1., *H. melpoda* vieill), butterfly finches (*H. phoenicotic* sws.), and Japanese titmice. Pseudotuberculosis in pigeons was reported by Dolfen (1916) and by Lesbouyries (1934). Reports on pseudotuberculosis in turkeys have appeared by Krage and Weisgerber (1924), Beck and Huck (1925), Lerche (1927a, 1927b), and Truche and Bauche (1929). Christensen (1927) isolated *Bact. pseudotuberculosis rodentium* (Pfeiffer) from pseudotuberculosis lesions of a variety of birds. Truche and Bauche (1933) have reported on an outbreak of pseudotuberculosis in fowls and pheasants, and according to Boquet (1937), they (Truche and Bauche, 1930) have also reported the disease in ducklings. Pseudotuberculosis in the swan was reported by Truche (1935). Urbain and Nouvel (1937) reported on

the disease in toucans (*Rhamphastos cuvieri* Gould and *R. ariel* Vig.). Beaudette (1940) reported on a case of pseudotuberculosis in a blackbird. Bacteriological investigations were made in the years 1938 to 1945 by Karlsson (1945) of 80 cases of pseudotuberculosis in domestic animals, 13 of which were found in birds, including 10 cases in turkeys.

Clapham (1953) observed pseudotuberculosis among stock-doves in Hampshire, England, during December, January, and February. In a search for the source of infection, *P. pseudotuberculosis* was isolated from a lark, a wood pigeon, a jackdaw, a rook, and a hare. The organism was also isolated from a rabbit in Hertfordshire and from two pigeons from Norfolk during this period. The authors had isolated the organism previously from gray partridge, pheasant, and bobwhite quail.

Van Dorssen (1952) isolated *P. pseudotuberculosis* from a bantam hen that had died. Multiple necrotic foci were seen in the liver and spleen. The organism was pathogenic for guinea pigs

Marthedal and Villing (1954) have observed seven outbreaks of pseudotuberculosis among pigeons in Denmark since 1950. There had been one case in a turkey and seven outbreaks in ornamental birds (canaries, snowbuntings, and waxwings). The organism isolated was *P. pseudotuberculosis rodentium*.

Thal (1954) studied 186 strains of *P. pseudotuberculosis*; of these, 33 were isolated from eight species of birds. All were biochemically identical; there were five serologically distinct groups. Most strains in group III produced thermolabile exotoxins, convertible to toxoids. Experimentally, anti-infection immunity was produced with live avirulent strains, thus protecting against infection with atoxic strains and subtoxic culture doses of toxic strains. Antitoxic immunity protected against toxin, and, to a degree, against infection with toxic strains, but not against infection with atoxic strains.

P. pseudotuberculosis was isolated from wild pheasants (*Phasianus colchicus*), partridge (*Perdix perdix*), and from a long-eared owl (*Asio o. otus*) by Borg and Thal (1961).

Etiology. The cause of this disease was first observed by Malassez and Vignal (1883) and was named *Bacterium pseudotuberculosis rodentium* by Pfeiffer (1890). Topley and Wilson (1936) and Breed *et al.* (1957) use the name *Pasteurella pseudotuberculosis*. In these two books and in the review of the literature on this disease by Beaudette (1940), there are complete descriptions of this organism. The earlier descriptions have been incomplete, making it difficult to identify newly isolated organisms on the basis of the available literature. Hutyra *et al.* (1938) claim that the designation, *Pasteurella pseudotuberculosis*, is a misnomer. However, this name is used here in conformity with the nomenclature accepted by the Society of American Bacteriologists.

The organism, which is not acid-fast, is a Gram-negative small rod 0.8 by 0.8 to 2.0 microns with rounded ends. Coccoid or long filamentous forms occur. It grows at 37° C. but optimally at 30° C. Motility develops at 30° C. or less and H antigens are produced if incubated at 22° C. Colonies after 24 hours at 37° C. are circular, 0.5 to 1.0 mm. in diameter, translucent and slightly yellow. Many types of media support growth. Acid but no gas is produced, sometimes only after prolonged incubation, from dextrose, maltose, mannitol, arabinose, xylose, rhamnose, salicin, and glycerol. Sucrose is sometimes fermented with acid production. It is H_2S+, indole—, methyl red+, catalase+, methylene blue is reduced, nitrates are reduced and ammonia is produced.

Occurrence. In the United States a definitely identified case of avian pseudotuberculosis in a blackbird was reported by Beaudette (1940) in New Jersey. Rosenwald and Dickinson (1944) studied several outbreaks of pseudotuberculosis in turkeys in Oregon. Mathey and Siddle (1954) isolated *P. pseudotuberculosis* from an 8-month-old tom turkey which died and showed typical lesions of pseudotubercu-

losis. There is a report by Kinyoun (1906) that may deal with this disease.

Kilian et al. (1962) isolated *P. pseudotuberculosis* from 9-month-old turkey hens. The organism was considered unusual in that it resembled *S. pullorum* on brilliant green agar, was agglutinated by *S. pullorum* antiserum, and after 24 hours incubation at 37° C. resembled *S. pullorum* by producing acid and no gas from dextrose and mannitol and failing to change lactose, sucrose, maltose, dulcitol, or salicin. Serum from sensitized guinea pigs, chickens, and turkeys which agglutinated hemologous antigen failed to agglutinate either *S. pullorum* or *S. typhimurium*.

Clark and Locke (1962) reported an epornitic of pseudotuberculosis in common grackles (*Quiscalus quiscula*), at a major icterid winter roost in Maryland. Mortality and morbidity were extensive and continued for several weeks until spring roost breakup. The occurrence of extensive infection in a large migratory bird population has major epizootiological significance.

In Europe, particularly in Germany and France, the disease must be fairly common, canaries and turkeys being the most commonly and severely affected. It usually occurs as a sporadic disease in individual flocks. One must remember that pseudotuberculosis often affects wild rodents, field hares and rabbits, and also domesticated rabbits and guinea pigs. Thus, such animals may be the source of infection in birds if direct or indirect contact is permitted.

Dissemination. The usual avenue of infection is the digestive tract. Skin injuries may also form portals of entry. Predisposing causes are apparently important since, as a rule, the only birds affected are those whose resistance has been lowered by inadequate feeding, exposure to cold, and worm infestation. Very young birds are particularly susceptible. During cold and wet weather in the fall, considerable losses may occur among young turkeys.

Pathogenesis. In susceptible birds the organism gains entrance to the blood stream through breaks in the skin or through the mucous membranes, perhaps mostly (but not necessarily exclusively) in the digestive tract. Thus, a bacteremia is established. Usually the bacteremic condition is of short duration, but the bacteria are not all destroyed. Some of them establish foci of infection in one or more organs such as the liver, spleen, lungs, intestines, and kidneys, giving rise to tubercular lesions. Such lesions have also been found in the mesentery and breast muscles.

Symptoms. The incubation period of artificial infection varies considerably with the virulence of the organism, the amount of inoculum, the avenue of introduction, and the host species. Sparrows and canaries are very susceptible and may die in 1 to 3 days from small doses of organisms injected subcutaneously or intramuscularly. Feeding of cultures is usually ineffective unless some intestinal inflammation is present to act as a predisposing influence. Canaries, given mustard seed for the purpose of causing intestinal irritation, have sickened 5 days after being given cultures by mouth, death resulting 2 days later. Judging from the various reports available, the incubation period may vary from 3 to 6 days in acute attacks and 2 or more weeks in chronic cases.

The symptoms also vary considerably. In very acute cases the birds may die suddenly without warning, or they may live a few hours or 2 to 3 days after showing the first symptoms. Such cases are usually marked by sudden appearance of diarrhea and the usual general manifestations of an acute septicemia. Usually, however, the course of this disease extends over 2 or more weeks, in which case the symptoms appear 2 to 4 days before death. In such cases the birds will show weakness, dull and ruffled feathers, and difficult breathing. Diarrhea is also a common symptom in such cases. Occasionally the disease will run a still more protracted course, when emaciation and extreme weakness or paralysis may be evident. Such manifestations as stiffness, difficulty in walking, droopiness, somnolence, constipation, and

discoloration of the skin have also been observed. In the early stages of the chronic form of the disease, the birds may eat normally, but the appetite is usually completely lost 1 or 2 days before death.

Anatomical changes. In highly acute cases the only changes observed are swelling of the spleen and enteritis. Subacute or chronic cases result in enlargement of the liver, spleen, kidneys, and lungs. Yellowish-white foci the size of millet seed may be found in the liver, spleen, lungs, kidneys, and breast muscles. There is usually severe enteritis, which is sometimes hemorrhagic. The serous cavities may sometimes contain more or less clear fluid.

Prognosis and diagnosis. The prognosis

is unfavorable. A definite diagnosis can be established only by isolation and identification of the organism since the symptoms and lesions are very similar to those of several other diseases such as fowl cholera, fowl typhoid, paratyphoid, spirochetosis, tuberculosis, and certain forms of the leukosis complex. In making a bacteriological examination, one must remember that the organism can be found in the blood in acute cases but must be sought in tissues in chronic ones.

Treatment and prevention. No medicinal treatment is available. Protective vaccination has not proved successful. Therefore, one must depend on the usual sanitary and hygienic procedures in combating this disease.

REFERENCES

Beaudette, F. R.. 1940. A case of pseudotuberculosis in a blackbird. Jour. Am. Vet. Med. Assn. 97:151

Beck, A., and Huck, W. 1925. Enzootische Erkrankungen von Truthühnern und Kanarienvögeln durch hämorrhagische Septikämie (Patacholera). Zentralbl. I. Bakt. I. Orig. 95:330.

Borg, K., and Thal, E. 1961. Pseudotuberkulosen (*Pasteurella pseudotuberculosis*) som zoonos. Svenska Läkartidn. 58:1923.

Boquet, P. 1937. Recherches expérimentales sur la pseudo-tuberculose des rongeurs. Ann. de l'Inst. Past. 59:541.

Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams & Wilkins Co., Baltimore. P. 399.

Bruner, A.J. 1906. Ein Beitrag zur Pseudotuberkulose der Vögel. Inaug. Dissert. Univ. Zürich.

Christensen, N. P. C. 1927. Pseudotuberkulose hos fugle foraarsaget af *Bacterium pseudotuberculosis rodentium* (Pfeiffer). Zentralbl I. Bakt. I. Ref. 87:186.

Clapham, P. A. 1953. Pseudotuberculosis among stock-doves in Hampshire. Nature 172:353.

Clark, M. C., and Locke, L. N. 1962. Case report: Observations on pseudotuberculosis in common grackles. Avian Dis. 6:506.

Dollen, H. 1918. Über eine pseudotuberkulöse, seuchenhafte Erkrankung bei Tauben. Inaug. Dissert. Hannover.

Freese 1907. Über seuchenhafte Erkrankungen mit septikämischem Charakter bei Kanarienvögeln. Deutsch. tierärztl Wochenschr. 15:561

Hutyra, F., Marek, J., and Manninger, R. 1938. Special Pathology and Therapeutics of the Diseases of Domestic Animals. Alexander Eger, Chicago. Vol. I. P. 684.

Karlsson, Karl-Fredrik. 1945. Pseudotuberkulos hos hönsfoglar. Skand. Vet. Tidskr. 35(11)673.

Kilian, J. G., Yamamoto, R., Babcock, W. E., and Dickinson, E. M.. 1962. An unusual aspect of *Pasteurella pseudotuberculosis* in turkeys. Avian Dis. 6:403.

Kimpson, J. J. 1906. Bird plague. Science 23:217.

Krage and Wesserger 1921. Eine Putenseuche mit Diplo-Streptobazillenbefund. Tierärztl. Rundschau. 30:508.

Letche 1927a. Über eine neue Seuche der Truthühner. Arch. f. Geflügelk. 1:311.

—— 1927b. Die "Paracholera" der Puten und ihre Beziehung zur Pseudotuberkulöse der Nagetiere. Zentralbl. I. Bakt. I. Orig. 104:493.

Lesbouyries, G. 1934. Pseudo-tuberculose du pigeon. Bul. de l'Acad. Vet. de France 7:103.

Malassez, L. 1884. Sur le microorganisme de la tuberculose Zoologique. Arch. de Physiologie Normale et Pathologique. Series 3, an.81.

—— and Vignal, M. 1883. Tuberculose zoologique (forme ou espèce de tuberculose sans bacilles). Arch. de Physiol. Normale et Path. Series 3, iv:369.

Marthedal, H. E., and Villing, G.J. 1954. Pasteurellosis and pseudotuberculosis among fowls in Denmark. Nord. Vet. Med. 6:651.

Mathey, W. J., Jr., and Siddle, P. J. 1954. Isolation of *Pasteurella pseudotuberculosis* from a California turkey. Jour. Am. Vet. Med. Assn. 125:482.

Messner and Scherm. 1908. Die infektiöse Nekrose bei den Kanarienvögeln. Arch. f. wiss. u. prakt. Tierheilk. 34:133.

Pfaff, F.: 1905. Eine infektiöse Erkrankung der Kanarienvogel. Zentralbl. f. Bakt. I. Orig. 38:275.

Pfeiffer, A.: 1890. Über die bacilläre Pseudotuberkulöse bei Nagethieren. Zentralbl. f. Bakt. I. Orig. 7:219.

Rieck, M.: 1889. Eine infektiose Erkrankung der Kanarienvogel. Deutsch. Zeitschr. f. Tiermed u. Vergleich. Path. 15:68.

Rosenwald, A. S., and Dickinson, E. M.: 1944. A report on *Pasteurella pseudotuberculosis* infection in turkeys. Am. Jour. Vet. Res. 5:246.

Schäfer, W.: 1939. Das Vorkommen des *B. pseudotuberculosis rod.* oder eines ihm ähnlichen Erregers bei Huhnerküken. Tierärztl. Rundschau 45:72

Thal, E.: 1954. Untersuchungen uber *Pasteurella pseudotuberculosis* unter besonderer Berucksichtigung ihres immunologischen Verhaltens. (Thesis) Berlingska Boktryckeriet, Lund, Sweden.

Topley, W. W. C., and Wilson, G. S.: 1936. The Principles of Bacteriology and Immunity. 2nd ed. Williams & Wilkins Co., Baltimore, p. 607.

Truche, C: 1935. Pseudo-tuberculose du cygne. Bul. de l'Acad. Vet. de France 8 278.

———, and Bauche, J.: 1929. La pseudo-tuberculose du dindon. Am. de l' Inst. Past. 43:1081.

———, and Bauche, J.: 1930. Contribution a l'étude de la pseudo-tuberculose des oiseaux. Bul. de l'Acad. Vet. de France 3:391.

———, and Bauche, J.: 1933. Le bacille pseudotuberculeux chez la poule et faisan Bul. de l'Acad. Vet. de France 6:43.

Truche, G., and Isnard, S.: 1937. Un nouveau cas de pseudo-tuberculose chez la poule. Bul. de l'Acad. Vet. de France 10.38.

Urbain, A., and Nouvel, J.: 1937. Epidemie de pseudo-tuberculose chez des toucans de cuvier (*Rhamphastos cuvieri* Gould) et des toucans ariel (*Rhamphastos ariel* Vig). Bul. de l'Acad. Vet. de France 10:188.

van Dorssen, C. A.: 1952. Pseudotuberculosis in a bantam fowl. Tijdschr. Diergeneesk. 77 297

van Heelshergen, T.: 1927. Pseudotuberculosis canariensis (Rodentium) bij Kanarievogels Tijdschr. v. Diergeneesk. 54:545.

v. Wasielewski, and Hoffman, W.: 1903. Über eine seuchenhafte Erkrankung bei Singvogeln. Arch. f. Hyg. 47:44.

Woronoff, A., and Sineff, A.: 1897. Zur pathologischen Anatomie und Bakteriologie der bacillaren Pseudotuberkulose. Zentralbl. f. allg. Path. u. path. Anat. 8 622.

Zeiss, H.: 1914. Über einige bei Tierkrankheiten gefundene Erreger aus der Gruppe der hämorrhagischen Septikamie und der Koligruppe. Arch. f. Hyg. 84:1.

Zurn, F. A.: 1884. Blätter f. Gefugeründt. Dresden, p. 326.

Zwick, W.: 1908. Untersuchungen uber eine Kanarienvogelseuche Zeitschr. f. Infekt.-Krenkh. d Haus. 4:33.

Tetanus

Tetanus is an acute infectious disease caused by the toxin of *Clostridium tetani* and is characterized by more or less persistent tonic spasms of some of the voluntary muscles resulting from an increased reflex irritability of the intoxicated motor nerve centers. The toxin is elaborated in infected wounds providing suitable conditions for growth and reproduction of the tetanus bacillus. Anaerobic wounds in which there is necrotic tissue or blood effusion are particularly favorable.

Occurrence. Birds are comparatively very resistant to tetanus. Knorr (1899) has shown that the lethal dose of tetanus toxin for fowl per kilogram of body weight is 200,000 times greater than that for the horse. This fact no doubt accounts for the scarcity of reports dealing with tetanus in birds.

Tetanus or tetanuslike disease in birds. Dreymann (1894) reported on a case of illness resembling tetanus in a turkey. Four to 5 days after having been bitten by a dog, this bird showed a clumsy and stiff gait, the neck was extended, the neck muscles were hard, and there was trismus. On the third day the entire body was rigid, the wings were held tightly against the body, the feathers were ruffled, the nictitating membranes and eyes protruded, there was almost complete trismus, and the bird died.

A case of tetanus in a young goose was described by Funck (1919). The symptoms were mainly trismus and difficult locomotion. The necropsy revealed a perforation of the stomach wall, caused by an iron wire, which in the author's opinion was the portal of entry.

Russell (1937), of Portsmouth, England, reported on a rapidly fatal case of illness in a young child, diagnosed as tetanus. An investigation revealed that the garden in

which the child played was fertilized exclusively with pigeon manure. Tetanus bacilli were recovered from two of four soil samples, two samples of dirt or litter from the pigeon loft, and from the excreta of one pigeon

On July 15, 1910, Dr J. H Scamman of the Angell Memorial Hospital, Boston, Massachusetts, wrote me in part as follows: "It is a six-months-old domestic bird (goose) and has all the appearances of lockjaw. It cannot open its jaws at all, and if they are forced apart, they close by themselves."

Another case of a tetanuslike disease in a goose was reported to the Michigan Agricultural Experiment Station, October 4, 1910. This goose was three months old and showed typical symptoms of tetanus.

In none of these four cases was there

any bacteriological work done to prove the identity of the disease.

Diagnosis. A positive diagnosis of tetanus can be established only by the isolation and identification of the organism. This is not always possible due to the difficulty in locating the focus of infection and the fact that tetanus bacilli often soon disappear from infected wounds. Remedial effects obtained by the administration of tetanus antitoxin would constitute evidence of some diagnostic value.

Treatment and prevention. Antitoxin given in large doses may be of some value in the very early stages of the disease in mammals. There is no work showing the effect of antitoxin treatment in birds. The infrequent occurrence of tetanus in birds makes special preventive measures superfluous.

REFERENCES

Dreymann 1894 Zwei seltene Falle von Tetanus beim Rind und Truthahn. Monatschr. f. Tierheilk. 5 75

Funck, E.: 1919. Starrkrampf bei einer Gans. Tierarzti. Rundschau 25:456.

Knorr, A: 1899 Die Tetanuserkrankung und ihre Bekämpfung. Monatschr. f. Tierheilk. 10:241

Russell, A. W · 1937. Pigeons as possible tetanus carriers. Brit Med. Jour. 2:1220.

Vibrio Infection

Vibrio infection is an acute infectious disease which resembles fowl cholera with respect to symptoms and gross pathology.

History. This disease was first reported by Gamaleia (1888) who observed it several times during the summer near Odessa. Krause and Windrath (1919) observed the disease in Germany in newly imported sunbirds (*Leiothrix luteus*). According to Hutyra et al. (1938), Czukas has observed it in Hungary in fattened geese.

Frazer (1961) isolated a strict anaerobic vibrio from liver and other tissues of an adult King penguin. The isolate failed to infect embryonating eggs and was indole, H_2S and catalase negative. It did not resemble either *Vibrio metchnikovi* or the vibrio responsible for avian vibrionic hepatitis.

Cause and dissemination. The cause is *Vibrio metchnikovi* (Gamaleia), also

called the paracholera vibrio. This organism is a small, bent, comma-shaped microbe that can be easily cultivated. It is very motile and is Gram-negative. Culturally and biochemically it resembles almost fully the vibrio of Asiatic cholera. These two organisms are also related antigenically as is shown by cross immunity. According to Czukas the organism infects birds that are weakened by poor hygienic conditions and by fattening. *Vibrio metchnikovi* is also pathogenic for pigeons and guinea pigs. Chicks can easily be infected per os. Older chickens and rabbits are very resistant. It appears that stagnant water and other materials contaminated with droppings of infected birds may serve as sources of infection.

Symptoms. In chickens the symptoms resemble those of fowl cholera. Vibrio infection is generally less acute and the body temperature is only slightly elevated, while

in fowl cholera it is usually high, e.g., 43° to 44° C. The birds observed by Gamalela sat crowded together and disinterested, with ruffled feathers. Diarrhea was a constant symptom. The birds usually died in 2 to 3 days. In the sunbirds the disease was so acute as to cause the death of several hundred birds in a few days.

Pathology. The organism causes enteritis and gastroenteritis which is sometimes hemorrhagic. It may enter the blood stream and give rise to necrotic processes in the liver and lungs. In young chickens and sunbirds the organism appears in the blood in large numbers, but in adult chickens the blood smears are mostly negative.

Diagnosis. A differential feature in the pathology of fowl cholera and vibrio infection in chickens is that the latter produces no or few petechial hemorrhages in the intestinal tract and only slight or no changes in the organs. A definite diagnosis can be made only by the demonstration of the organism in the blood and preferably by the isolation and identification of the vibrio. Cultures should be made from the blood and organs showing lesions. The bacteriological diagnosis is complicated by the fact that the organism is generally confined to the lumen of the intestinal tract.

Treatment and prevention. Modern drugs have not been evaluated for either prophylaxis or therapy of the disease. The considerable number of drugs known to be effective against other vibrionic infections suggests possible activity of some of these drugs against *Vibrio metchnikovi*. Prevention must depend on the usual sanitary measures employed in the control of diseases of similar epidemiological nature.

REFERENCES

Fraser, G: 1961. Isolation of an anaerobic vibrio from a King penguin (*Aptenodytes longirostris*). Avian Dis. 5:243.
Gamalela, N: 1888. Sur l'étiologie du choléra des poules. Ann. de l'Inst. Past. 2:510.
Hutyra, F., Marek, J., and Manninger, R.: 1938. Vibrio cholera in fowls (Gastro-enteritis Cholerica Avium). Pathology and Therapeutics of the Diseases of Domestic Animals. Alexander Eger, Chicago. Vol. 1, P. 122.
Krause, W., and Windrath, H.: 1919. Über eine durch einen Vibrio veranlasste Seuche der Sonnenvogel (*Leiothrix luteus* L., chinesische Nachtigall). Berliner tierarztl. Wochenschr. 35:468.

Avian Vibrionic Hepatitis

Avian vibrionic hepatitis is a contagious disease of chickens which ranges in clinical course from acute to chronic. All ages of chickens are susceptible, but the disease is diagnosed chiefly in semimature and mature stock.

Tudor (1954) reported a disease, presumably avian vibrionic hepatitis, and stated that the disorder was observed by Beaudette and Hudson as early as 1933. Marthedal (1953) described a severe degenerative hepatitis occurring chiefly in birds under three months of age, usually accompanied by typhlitis and splenomegaly. No infectious agent was demonstrated. Delaplane et al. (1955) were the first to report an infectious agent as being responsible for the disease. Hofstad (1956) and Winterfield and Sevoian (1957) also reported the occurrence and infectious nature of the disease. Hofstad et al. (1958) and Peckham (1958) simultaneously classified the causal agent as a vibrio.

Distribution. The disease has been identified in the major poultry producing areas of the United States and Canada, in Japan (Miura, 1962), in Germany (Fritzsche and Gerriets, 1962), and in Scotland (Wilson, 1963). Reports of the northeastern state diagnostic laboratories for 1957 through 1961 (Angstrom, 1958, 1959, 1960, 1961, 1962) show that the annual incidence of avian vibrionic hepatitis ranged from 1.26 to 1.85 per cent of all avian cases submitted for diagnosis. Most of the diagnoses were made in flocks over 20 weeks of age
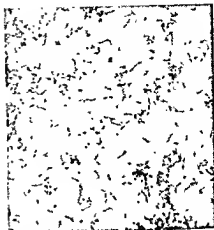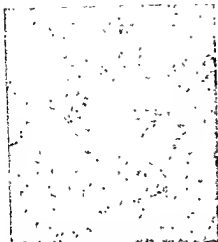
FIG. 14.1 — (Left) Giemsa-stained smear of sedimented broth culture of vibrio (698 — 18th transfer, 96 hr.). ×885. (Right) Giemsa-stained smear of sedimented broth culture illustrating spiral forms (698 — 17th transfer, 6th day). ×885. (Hofstad, Iowa State University)
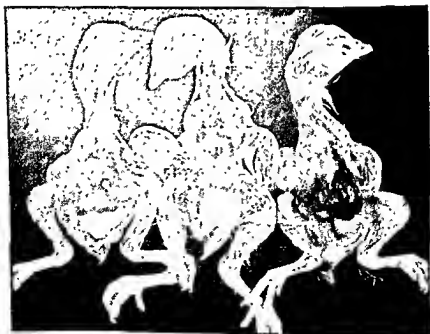
FIG. 14.2 — Chicken embryos at 19 days of age. Normal embryo in center. Two embryos, inoculated with hepatitis agent at 11 days of age, have liver enlargement and necrosis. (Hofstad et al., 1958, Avian Diseases.)

with very few being made at less than 4 weeks of age.

Etiology. The causative agent was first isolated in the yolk sac of 5- to 7-day embryonating chicken eggs by Delaplane et al. (1955). Peckham (1958) and Hofstad et al. (1958) were the first to identify the agent as a vibrio. It is a micro-aerophilic, Gram-negative, motile, comma or S-shaped rod that is occasionally spiral in form (Fig. 14.1). Older cultures on agar consist chiefly of coccoid forms.

Most embryos infected via the yolk sac at 5 to 7 days of age die between the 2nd and 6th day after injection. The vibrio develops in high concentration in the yolk of infected embryos regardless of the route of infection; amniotic and allantoic fluids contain few or no vibrios (Winterfield et al., 1958). Grossly visible liver necrosis and enlargement of the spleen occurs in embryos inoculated into the allantoic sac at 10 to 15 days of age (Fig. 14.2) (Hofstad et al., 1958) as well as in late deaths following yolk sac inoculation.

The organism has been grown in cell-free mediums. Hofstad (1956) grew the agent in a chicken-serum enriched, chicken infusion broth. Peckham (1958) isolated the organism on chicken infusion agar incubated in a 10 per cent CO_2 atmosphere as well as by inoculating a few drops of bile from infected chickens on blood agar plates incubated at 37.5° C. in a Brewer anaerobic jar containing 10 per cent CO_2, 63 per cent methane, and 27 per cent air. The following characteristics of an isolate examined in some detail were reported by Peckham (1958): glucose −, xylose −, mannitol −, lactose −, sucrose −, maltose −, MacConkey + light, S.S. −, hemolysis −, citrate −, urea −, nitrate −, indole −, H_2S TSI paper 2 + butt −, MR/VP − / −, gelatin −, litmus milk −, spores −. O_2 requirements microaerophilic, temperature 37° to 42° C., pigment slight yellow, oxidase +, motility +, catalase 2 +. Whenham et al. (1961) secured satisfactory growth in semisolid thiol medium with consistent fermentation of dextrose when added; an indicator was added after 24 or 48 hours incubation. Occasional slight fermentation of lactose, maltose, and mannitol was secured with the same system but not of sucrose or dulcitol. H_2S was not formed from Kligler's iron agar.

The agent is sensitive to streptomycin, dihydrostreptomycin, tetracycline, oxytetracycline, chlortetracycline, magnamycin, furazolidone, neomycin, erythromycin, sulfamethazine, sulfaquinoxaline, and thallium acetate. It is resistant to bacitracin, polymixin B sulfate, and chloramphenicol and penicillin except in large amounts. Sensitivity differences between strains probably occur (Winterfield et al., 1958; Hofstad et al., 1958; Whenham et al., 1961).

The bacterium is filterable through a Selas 02 filter but is retained by the 03 Selas filter (Hofstad et al., 1958). It passed through the 0.45 millipore filter but was retained by the 0.30 membrane filter as well as by Seitz EK filters (Winterfield et al., 1958).

Hofstad et al. (1958) found the organism viable after storage in infected embryo yolk for 2 years at −25° C. Peckham (1958) found the agent viable 20 months after lyophilization. Winterfield et al. (1958) found infective yolk viable after 2 but not 3 weeks at 37.5° C. At 25° C. another strain remained viable for only 12 days.

Peckham (1958) noted that some isolates were readily adapted to culture on artificial media, whereas other isolates were difficult to subculture and in some cases were lost after 3 or 4 transfers. Winterfield et al. (1958) found that isolates lost pathogenicity after serial yolk sac passage.

Hosts. Natural infections have been recorded only in chickens. Day-old poults were experimentally infected by Peckham (1958) and Winterfield et al. (1958). The latter group reported death, with resolation from the liver of a 4-month-old rabbit 5 days post intraperitoneal injection. Infection was not established in adult hamsters or in mice injected intraperitoneally. Peckham (1958) injected white Swiss mice intracerebrally, produced nervous symp-

ions with some colonies and re-isolated the organism.

Signs and mortality. The usual clinical signs in affected flocks indicate a chronic infection. Affected chickens exhibit listlessness, a scaly drawn comb, loss of flesh, and frequent diarrhea. Peckham (1958) and Sevoian et al. (1958) observed a lag in egg production in affected pullets and as much as a 25 to 35 per cent drop of production in laying flocks. In laying flocks signs of an acute infection may occur with mortality of birds in excellent flesh which have laid eggs within the past 48 to 72 hours. Moore (1958), using a laparotomy technique, observed that some birds with signs and lesions spontaneously recovered. Mortality was reported as high as 5 per cent by Peckham (1958) and 10 to 15 per cent by Sevoian et al. (1958).

Pathology. The liver is the primary site of gross lesions. Usually the liver is firm,

having irregularly shaped, grayish-white areas on the surface (Fig. 14.3). In more acute cases the liver is swollen, congested, and studded with necrotic areas. Hemorrhagic areas may also be present, giving the liver a mottled appearance. Massive subcapsular hemorrhages may occur which occasionally rupture and cause death from frank hemorrhage. In chronic cases, varying degrees of ascites and hydropericardium may be present. The entire liver is not always involved; only part of a lobe may show degenerative areas.

The microscopic lesions have been studied extensively by Sevoian et al. (1958). Sections of liver may reveal generalized congestion, localized hemorrhages, and areas of necrosis. Infiltration of the liver with lymphocytic and heterophilic cells with bile duct proliferation is common. Figure 14.4 illustrates the type of lesion which may be found. The heart of young chickens may be edematous and contain areas infiltrated with mononuclear cells. Embryo livers exhibit focal necrosis, occasional aggregates of heterophils accompanied by bile duct proliferation, bile stasis, and regenerating liver cord cells about the hepatic vessels (Casorso and Jungherr, 1958).

Transmission. Delaplane et al. (1955) successfully infected day-old chicks by feeding as well as by subcutaneous and intraperitoneal inoculation. Sevoian et al. (1958) confirmed these findings and found lesions as early as 48 hours after injection of 2-day-old chicks, with hepatic lesions being most pronounced between 3 and 12 days. Signs were not usually prominent and frequently were absent. In older chickens gross lesions were not seen until 5 to 15 days after inoculation, with lesions persisting for at least 9 weeks at which time the vibrio was still recoverable.

Sevoian and Calnek (1959) incubated over 1,200 eggs from experimentally infected chickens and found 66 per cent fertile, of which 13 per cent died by the twentieth day of incubation. The agent was not isolated from yolk sacs of the latter by culture on blood agar and embryo

FIG. 14.3 – Liver of a field case illustrating irregularly shaped degenerative areas found in avian vibrionic hepatitis. (Bennett, Iowa Vet. Diag. Lab.)
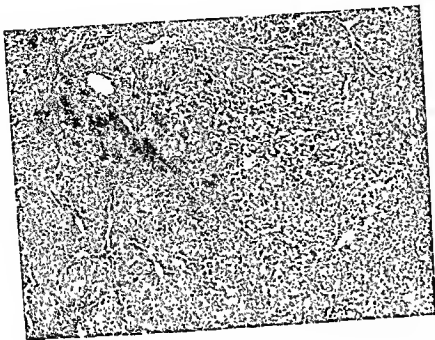
FIG. 14.4 — Liver section of field case of avian hepatitis. Hemorrhage around central vein. Light area to right of center is devoid of liver cord cells and contains erythrocytes, heterophiles, and some lymphocytes. X96. (Hofstad, Iowa State University)

inoculation. Hofstad et al. (1958) inoculated the allantoic sac of 15-day-old embryos; 34 of 52 hatched, some of which had liver necrosis although the birds appeared clinically normal. Narotsky and Taylor (1958) found gross and microscopic lesions indistinguishable from those produced by the vibrio in chicks hatched from infected flocks. Limited isolation attempts were negative. Additional work appears necessary before the possible role of egg transmission of the disease can be determined. The organism is presumably excreted in the feces; ingestion of material contaminated by feces is assumed to be a major means of transmission.

Diagnosis. Several other diseases bear a close clinical and gross pathological similarity and must be eliminated in differential diagnosis. Most prominent of these are pullorum disease, fowl typhoid, staphylococcosis, and leukosis.

A diagnosis based on isolation and characteristic gross and microscopic pathology is preferred. Considerable caution is indicated as: 1. isolation is not always successful, 2. slight histological changes may not be sufficiently characteristic to be diagnostic, and 3. presence of the organism

with characteristic pathologic changes does not necessarily indicate a flock problem. Thorough appraisal of the history as well as thorough examination of multiple specimens helps to overcome these difficulties.

The vibrio may be isolated from many sites including the heart, liver, spleen, pericardial fluid, kidneys, and bile. Bile is preferred and may be aspirated from the gallbladder and dropped without germinating on a blood agar plate. Plates are incubated under 10 per cent CO_2 for 72–96 hours. Demonstration of typical Gram-negative vibrios in resulting growth is usually considered adequate. Injection of bile in 0.1 ml. amounts into the yolk sac of 5- to 7-day-old embryos is a superior method of isolation (Sevoian and Calnek, 1959). Bacterial contaminants may be controlled by the use of bacitracin and polymixin B (Winterfield et al., 1958). Yolk material from embryos that succumb may then be used for direct plating or repassage. Motile vibrios may at times be demonstrated in bile as well as in yolk material from infected embryos; only positive findings have significance (Whenham et al., 1961). It is worthwhile to point out the possibility that other as yet unrecognized

vibrios with or without pathologic significance may exist in bile or other organs of the bird. Lack of simple means of precise identification of the vibrio increases the importance of auxiliary diagnostic criteria.

Chicks are particularly susceptible during the first days of life and serve as an excellent means of confirming isolations. Injection into the yolk sac or peritoneal cavity usually produces lesions within 3–5 days. However, chicks seldom appear clinically ill and must be sacrificed to demonstrate infection. Focal hepatic necrosis and hydropericardium are usually exhibited.

No serologic tests are available for diagnosis.

Treatment. It has already been indicated under the section on etiology that the organism is sensitive to a large number of antibiotics and to several chemotherapeutic agents. Winterfield et al. (1958) reported extensive prophylactic and therapeutic trials in chicks. Prophylactic activity was demonstrated by chlortetracycline, oxytetracycline, and furazolidone administered in the feed, by sulfaquinoxaline and sulfamethazine in water, and by streptomycin by injection. Oxytetracycline, furazolidone, and streptomycin appeared superior to the others tested. On a therapeutic basis in chick tests, furazolidone and streptomycin appeared more effective than the others. Streptomycin at a dosage of 5 mg. intramuscularly per chick was compared to 0.022 per cent and 0.044 per cent

furazolidone in the feed. At the lowest level (200 gm. per ton) furazolidone was more effective than streptomycin. Sevoian and Calnek (1959) compared the prophylactic activity of 300 gm. per ton of furazolidone in the feed for 5 consecutive days to a 250 mg. single injection of dihydrostreptomycin in adult White Leghorn hens. A second trial compared the same dosage of dihydrostreptomycin to 250 gm. per ton furazolidone fed for 5 consecutive days to mature Rhode Island Red hens. A high degree of efficiency was indicated following each of the treatments. Relapse or reinfection was noted in the trials and has also been noted with some frequency in field flocks. Furazolidone in the feed ration at 0.022 per cent (200 gm. per ton) for a 10- to 12-day period is recognized as a standard treatment. Streptomycin by injection is also used at a level as high as 50 mg. per pound of body weight.

Prevention and control. Moore (1958) and Winterfield et al. (1958) were unable to demonstrate immunity in infected chickens. Although limited trials so far have been negative, egg transmission cannot be excluded. Until more is known concerning the source of infection, means of transmission, and reservoirs of infection, prevention must be based on sound management practices and sanitation. Treatment with furazolidone or streptomycin offers limited control capability; the potential for prophylaxis by continuous low level administration of drugs has not been adequately explored.

REFERENCES

Angstrom, C. 1: 1958. Report of the committee on nomenclature and reporting of diseases. Northeastern conference on avian diseases Avian Dis 2.543
———: 1959. Report of the committee on nomenclature and reporting of diseases. Northeastern conference on avian diseases. Avian Dis. 3·488
———: 1960. Report of the committee on nomenclature and reporting of diseases. Northeastern conference on avian diseases. Avian Dis. 4 549
———: 1961. Report of the committee on nomenclature and reporting of diseases. Northeastern conference on avian diseases Avian Dis. 5:463
———: 1962. Report of the committee on nomenclature and reporting of diseases. Northeastern conference on avian diseases. Avian Dis 6 516
Casorso, R. D., and Jungherr, E. L.: 1958 Abstract. The pathology of chicken embryo inoculated with certain avian viruses. Avian Dis. 2:542.
Delaplane, J. P., Smith, H. A., and Moore, R. W.: 1955. An unidentified agent causing a hepatitis in chickens. The Southwestern Vet. 8.356.
Fritsche, E., and Gerriets, E.: 1962. Geflügelkrankheiten. Paul Parey in Berlin and Hamburg.
P 316

Hofstad, M. S.: 1956. Report of Progress in Veterinary Medical Research. Iowa State College, p. 15.

——, McGehee, E. H., and Bennett, P. C.: 1958. Avian infectious hepatitis. Avian Dis. 2:358.

Marthedal, H. E.: 1953. Hepatitis in chickens, presumably of non-contagious character. Proc. Internat. Vet. Cong., Stockholm, 1953. 2:1067.

Miura, M.: 1962. Personal communication.

Moore, R. W.: 1958. Studies of an agent causing hepatitis in chickens. Avian Dis. 2:39.

Narotsky, S., and Taylor, J. R. E.: 1958. Abstract. Clinical evidence suggesting the possibility of egg transmission of avian hepatitis. Avian Dis. 2:541.

Peckham, M. C.: 1958. Avian vibrionic hepatitis. Avian Dis. 2:348.

Sevoian, M., and Cainek, B. W.: 1959. Avian infectious hepatitis. III. Treatment of chickens in egg production. Avian Dis. 3:302.

——, Winterfield, R. W., and Goldman, G. L.: 1958. Avian infectious hepatitis. I. Clinical and pathological manifestations. Avian Dis. 2:3.

Tudor, T. C.: 1954. A liver degeneration of unknown origin in chickens. Jour. Am. Vet. Med. Assn. 125:219.

Whenham, G. R., Carlson, H. C., and Aksel, A.: 1961. Avian vibrionic hepatitis in Alberta. Canad. Vet. Jour. 2:3.

Wilson, J. E.: 1962. Personal communication.

Winterfield, R. W., and Sevoian, M.: 1957. Isolation of a causal agent of an avian hepatitis. Vet. Med. 52.273.

——, Sevoian, M., and Goldman, C. L.: 1958. Avian infectious hepatitis. II. Some characteristics of the etiologic agent. Effect of various drugs on the course of the disease. Avian Dis. 2:19.

Borrelia Anserina Infection (Spirochetosis)*

The disease, caused by *Borrelia anserina* (Breed *et al.*, 1957), was first described by Sakharoff (1891). The organism, which he named *Spirochaeta anserina*, was responsible for an extensive epornitic among geese in the Caucasus. Other names which have been used are *Spirochaeta gallinarum*, *Spirochaeta anatis*, and *Treponema anserina*.

Distribution. The infection has been identified in many countries of the world, but apparently not further north of the equator than 54 to 60 degrees parallel (Zuelzer, 1936). Its distribution is similar to that of the tick *Argas persicus* Oken 1818 which is its major vector. Although the tick is prevalent in the southern part of the United States, the disease is not commonly found in this region. Hoffman and Jackson (1946) identified in turkeys the first authentic case of avian *B. anserina* infection in the United States. It was also identified in turkeys in California by McNeil *et al.* (1949) and by Loomis (1953). Francis (1956) and Rokey and Snell (1961) reported the disease in chickens in New Mexico and Arizona respectively. Burroughs (1947) identified the disease in a cockerel used to feed ticks (*A. persicus*) obtained from Texas. Mathey and Siddle (1955) reported a natural infection in Mongolian pheasants on a California game farm. Mathey and Zander (1955) reported a spirochete isolated from cecal nodules of chickens which is thought not to be *B. anserina* but probably the same as the unnamed organism reported by Steinhaus and Hughes (1947).

Etiology. *B. anserina* is reported to vary in length from 6 to 30μ with great variations noted within preparations. McNeil *et al.* (1949) report an average length of 14μ (7 to 21μ) with 6 spirals. The organism is motile, stains readily with aniline dyes (in contrast to *Leptospira* and *Treponema*), reproduces by binary fission, and is soluble in 10 per cent ox bile and 10 per cent saponin.

Hosts. The organism is infectious for a wide variety of birds. Literature summaries by Knowles, *et al.* (1932) and by El Dardiry (1945), indicate such discrepancy between the findings of different investigators as to the degree of susceptibility of different species. El Dardiry (1945) suggests that genetic resistance may be an explanation. Antigenic differences

quite likely occur and possibly not all isolates have equal pathogenicity for all hosts.

Mild infections have occasionally been produced in rabbits, but mice, rats, guinea pigs, dogs, mules, horses, and sheep have been found refractory (El Dardiry, 1945; McNeil et al., 1949).

Transmission. Marchoux and Salimbeni (1903) first demonstrated the tick *A. persicus* to be a primary vector of *B. anserina*. The infected tick lays infected eggs which hatch into infected larvae. Larvae and nymphs apparently are more infective than are adults (Sreenivasan and Sankaranarayan, 1943). The review by El Dardiry (1945) indicates that *A. mineatus* and *Ornithodorus moubata* may also serve as vectors. Hungerford and Hart (1937) demonstrated that the red mite *Dermanyssus gallinae* may be a vector; *culex* mosquitoes have also been incriminated (Zuelzer, 1936; El Dardiry, 1945).

The disease may also be transmitted in the absence of vectors. Sreenivasan and Sankaranarayan (1943) reported transmission between chickens by cohabitation. The infections in California turkeys (Hoffman and Jackson, 1946; McNeil et al., 1949; Loomis, 1953) were not traced to vectors. Infected droppings were believed to be the major means of transmission within the flocks. A California strain of *B. anserina* was maintained in *A. persicus* by Hsiang and Packchanian (1951).

Neither egg transmission of the organism nor carriers have been reported.

Signs and lesions. The incubation period is largely dependent upon dosage, and may be as little as 1 day. The incubation period of natural infections ranges from 3 to 8 days. A marked rise in body temperature is accompanied or soon followed by the appearance of *B. anserina* in peripheral blood. Acutely affected birds show cyanosis of the head, marked depression, increased thirst, and diarrhea. Subacute or chronic cases are reported. A mortality of 50–60 per cent may occur chiefly between 3 to 15 days post infection. Although young birds are generally con-

sidered to be more susceptible than old birds, Rokey and Snell (1961) found the opposite to be true.

Gross lesions are found chiefly in the spleen and liver. In terminal cases the spleen is enlarged as much as six times normal and is mottled as a result of hemorrhage. The liver is enlarged, frequently bile stained, friable, and studded with small necrotic foci. The heart may present a parboiled appearance. A mucoid enteritis is often present. Dehydration and emaciation is marked. Extreme weakness, or possibly paralysis, may be seen in chronic infection.

Histopathological changes in the chicken have not been extensively studied. Rokey and Snell (1961) describe the changes as follows:

"In the kidneys, collecting tubules had undergone coagulation necrosis. Capillaries were engorged. Hemorrhagic infarcts in various stages of resolution were encountered. Lymphocytic infiltration of interstitial tissue was a common finding.

"In the liver, there were subcapsular hemorrhages. Multiple areas of focal necrosis and a cellular infiltration of the hepatic triads were seen. The cellular infiltration consisted of lymphocytes and large phagocytic cells. The venous system had a characteristic lymphocytic proliferation zone. The large bile ducts were surrounded by a wide zone of lymphocytic proliferation. Fatty degeneration of varying degrees was observed.

"Large areas of hemorrhage were prominent in the spleen, and necrosis was observed in centers of reticular cell groups. The general splenic structure was intact, but an active proliferation of lymphocytic elements gave the splenic structure an abnormal appearance.

"Localized nests of lymphoid cells were seen between the cardiac muscle fibers. There was little apparent damage to the cardiac muscle.

"Congestion, hyperemia, and pneumonic areas were observed in the lungs. In some of these areas, lymphocytic infil-

trations completely obliterated the normal lung structure."

Quite similar histopathological changes have been described in turkeys by McNeil et al. (1949).

Diagnosis. Diagnosis is ordinarily dependent upon demonstration of the organism. It may be demonstrated in blood smears by the Giemsa technique or may be seen in wet mounts by dark-field illumination. Appreciable numbers are present within the first several days of the disease, but disappear at crises after clumping in large aggregates as a result of the development of agglutinating antibodies. Death may be due to the formation of emboli by the agglutinated organisms (Lavaditi, 1904; DeLamater et al., 1952). Cultivation on artificial mediums has met with mixed success and has been summarized by McNeil et al. (1949) and Saurino and DeLamater (1952). Knowles et al. (1932) grew the organism in fertile chicken eggs. McKercher (1950) secured improved results by yolk sac injection of 6-day embryos. In the latter technique, material must be harvested from live embryos in a manner to allow collection of chorioallantoic fluid and embryonic blood, as B. anserina is found chiefly in the latter. Such harvests were demonstrated to remain viable for 2–3 weeks when held at 4° C.

El Dardiry (1945) maintained B. anserina in virulent form for 30 days in a refrigerator after separation from clotted blood taken from infected chickens. This method maintained virulence better than if maintained in citrated blood (Sreenivasan and Sankaranarayan, 1943). McNeil et al. (1949) found spleen, heart, and liver infectious after storage at 32° F. for 31 days.

Nobrega and Reis (1947) reported finding the organism by dark-field illumination in heart blood of 85 per cent of chickens which died after experimental infection. They described a method, given in more detail in Chapter 41, of testing for antibodies which greatly improved diagnostic accuracy in those specimens in which the organism was not found.

Lesions are not diagnostic unless the organism is demonstrated in tissues by an appropriate stain such as Levaditi or by the slow method of Giemsa.

Treatment and control. Early work on immunity development and immunization methods was reviewed and extended by El Dardiry (1915). A solid immunity develops in survivors; relatively small amounts of serum from such birds may be used for passive immunization. Active immunization has been accomplished with antigens prepared and used by a variety of means including formalin inactivation of blood or emulsified liver and spleen, infectious blood inactivated by immune serum, and by administering immune serum prior to active infection. Immunity by several of these methods has lasted for at least 18 months. Nobrega and Reis (1941) prepared a vaccine by infecting 12-day-old chicken embryos. They harvested the embryo, membranes, and amniotic fluid after 5 days, diluted with saline to 30 ml. per egg, and inactivated with 0.5 per cent formalin. The suspension was filtered after 24 hours of refrigeration. A 25 per cent dilution of the stock suspension was used intramuscularly in a 1 ml. dose. They considered this vaccine to be equal in efficacy and more economical than that made from blood or organs of chickens.

A variety of drugs have been demonstrated to have therapeutic value. Earlier reports were summarized and additional compounds were tested by Packchanian (1950) and by Hsiang and Packchanian (1951). The following compounds have been proven to be effective in vivo: atoxyl, salvarsan, myosalvarsan, "spirocid," neoarsphenamine, mapharsen, tryparsamide, 4-carbamidophenyl di (1'-carboxyphenyl 2' thio) arsenite, and the antibiotics penicillin G, oxytetracycline, chlortetracycline, bacitracin, chloromycetin, streptomycin, and dihydrostreptomycin. Of the antibiotics, Hsiang and Packchanian (1951) found oxytetracycline to be most effective. A single injection of 0.1 mg. per 50 gram chick was found to be curative. Compa-

rable results were secured with 1 mg. chlortetracycline or bacitracin and with a 10 mg. dosage of streptomycin or penicillin.

Control or eradication of vectors will substantially aid in preventing the disease. Spread of infection within a flock should be minimized by sanitary disposal of cadavers and droppings.

REFERENCES

Breed, R. S., Murray, E. C. D., and Smith, N. R.: 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams and Wilkins Co., Baltimore. P. 698.

Burroughs, A. L.: 1947. Fowl spirochaetosis transmitted by *Argas persicus* (Oken) 1818, from Texas. Sci. 105:577.

DeLamater, E. D., Saurino, V. R., and Urbach, F.: 1952. Studies on the immunology of spirochetosis. I. Effect of cortisone on experimental spirochetosis. Am. Jour. Syph. Gonor. and Ven. Dis. 36:127.

El Dardiry, A. H.: 1945. Studies on avian spirochaetosis in Egypt. Ministry of Agriculture, Egypt, Tech. Sci. Service Bul. 243:1.

Francis, D. W 1956. A case of fowl spirochetosis in New Mexico. (Abstr.) Poultry Sci. 55:1142.

Hinshaw, W. R., and McNeil, E.: 1946 Studies on a spirochete found in the blood of sick turkeys. Jour. of Bact. 51:599.

Hoffman, H. A., and Jackson, T. W.: 1946. Spirochaetosis in turkeys. Jour. Am. Vet. Med. Assn. 109:481.

Hsiang, Chin-Min, and Packchanian, A.: 1951. A comparison of eleven antibiotics in the treatment of *Borrelia anserina* infection (spirochetosis) in young chicks. Tex. Rep. Biol and Med. 9 34

Hungerford, T. G., and Hart, L.: 1937. Fowl tick fever (spirochaetosis), also transmitted by common red mite. Agr. Gaz. N. S. Wales 48:591.

Knowles, R., DasGupta, B. M., and Basu, B. C.: 1932. Studies in avian spirochaetosis. Indian Med. Res. Mem. 22:1.

Lavadin, C.: 1904. Contribution a l'etude de la spirillose des poules. Ann. Inst. Pasteur 18.129

Loomis, E. C.: 1953 Avian sprochetosis in California turkeys. Am. Jour. Vet, Res 14.612.

McKercher, D. G.: 1950. The propagation of *Borrelia anserina* in embryonated eggs employing the yolk sac technique. Jour. of Bact. 59.446.

McNeil, E, Hinshaw, W. R., and Kissling, R. E.: 1949. A study of *Borrelia anserina* infection (spirochetosis) in turkeys, Jour. of Bact. 57:191.

Marchoux, E., and Salimbeni, A.: 1903. La spirillose des poules. Ann. Inst. Pasteur 17:569.

Mathey, W. J., and Siddle, P. J.: 1955. Spirochetosis in pheasants. Jour. Am. Vet. Med. Assn. 126:123

———, and Zander, D. V.: 1955 Spirochetes and cecal nodules in poultry Jour. Am. Vet. Med. Assn 126 475

Nobrega, P., and Reis, J.: 1941. Producao de vacina contra a espiroquetose aviaria em ovos embrionados. Arq Inst. Biol. São Paulo 12.87.

———, and Reis, A. S.: 1947. O diagnostico da espiroquetose aviaria em animais mortos. Arq. Inst. Biol São Paulo 18.91.

Packchanian, A.: 1950. Chemotherapy of *Borrelia anserina* infection (spirochetosis) in young chicks. Tex. Rep. Biol. and Med. 8.78.

Rokey, N. W., and Snell, V. N.: 1961. Avian spirochetosis (*Borrelia anserina*) epizootics in Arizona poultry. Jour. Am. Vet. Med. Assn. 138 648.

Sakharoff, M. N.: 1891 *Sprochaeta anserina* et la septicemie des oies. Ann. Inst. Pasteur 5:564.

Saurino, V. R., and DeLamater, E. D.: 1952. Studies on the immunology of spirochetosis. II. Immunologic relationships of *Treponema pallidum* and *Borrelia anserina*. Am. Jour. Syph. Gonor and Ven. Dis. 36:355.

Sreenivasan, M. K., and Sankaranarayan, N. S. 1943. Spirochaetosis of fowls in India. Brit. Vet Jour. 99 208–214, 236–243, 255–258.

Steinhaus, E A., and Hughes, L, E.: 1947. Isolation of an unidentified spirochete from hen's eggs after inoculation with liver tissue from hens. U.S. Publ. Health Reps. 62:509.

Zuelzer, M.: 1936. *Culex*, a new vector of *Spirochaeta gallinarum*. Jour. Trop. Med. and Hyg 39.204.

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15

Listeriosis, Botulism, Erysipelas, and Goose Influenza

Avian Listeriosis

Sporadic cases or outbreaks of septicemia caused by the bacterium *Listeria monocytogenes* have been reported in chickens and other birds by a number of workers. The same organism also causes disease in mammals, including man.

Listeria was first isolated by Murray *et al.* (1926) from an outbreak of disease in laboratory rabbits and guinea pigs in England. It was first found to be the cause of an encephalitis of sheep by Gill in 1931 in New Zealand, and has since been described from ruminants by a number of investigators.

Listeria is found throughout the world. It has been found to cause spontaneous disease or be present in at least 35 species of mammals, 17 of birds, pond reared trout, ticks, crustacea, stream water, mud, sewage and silage (Gray, 1963). Infected mammals include the domestic rabbit, hare, guinea pig, chinchilla, gerbil, lemming, vole, sheep, ox, goat, deer, pig, horse, dog,

cat, skunk, fox, mink, raccoon, ferret, and man. A great many papers have been written on this organism. Among recent reviews are those of Seeliger (1961), Gray (1958, 1963), Jones and Woodbine (1961), Kampelmacher (1962), and the Office International des Épizooties (1961).

Listeria was first isolated from chickens in New Jersey by Ten Broeck in 1932 according to Seastone (1935), who also reported on the disease. Gray (1958) listed 43 other reports from chickens in the United States, Canada, England, France, Germany, Sweden, the Netherlands, Belgium, Argentina, Finland, Czechoslovakia, Russia, Australia; it has also been found in chickens in Italy (Sparapani, 1916).

Listeriosis has been found in geese in Germany, Hungary, Russia, and Ceylon; in ducks in Germany and France; in turkeys in the United States; in pigeons in Czechoslovakia and the Netherlands; in canaries in Canada, Norway, and the Netherlands; in a parrot in Czechoslovakia; in a blue eagle in the Leipzig zoo; in the capercaillie
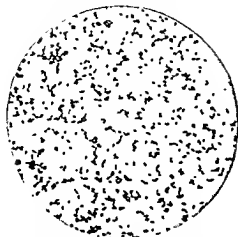
FIG. 15.1 – Listeria monocytogenes from chicken. Gram stain. ×990.

(*Tetrao urogallus*) in Sweden; in the partridge in France; and in a snowy owl in Canada (Gray, 1958; Levine, 1959; Jones and Woodbine, 1961). More recently, Listeria has been isolated from canaries and chickens in the Netherlands by Kampelmacher (1962) and Kampelmacher and Jansen (1961); from chickens, partridges, pheasants, a duck, a pigeon, and a canary in France by Lucas (1961) and Lucas et al. (1962); from poultry in Sweden by Nilsson and Karlsson (1959); from the chicken, goose, crane, and ptarmigan in Finland by Stenberg (1961); from chickens in Germany by Train (1961, 1962); and from the diamond dove (*Geopelia cuneata*), Swainson's lorikeet (*Trichoglossus novae-hollandiae*), and canary in the Netherlands by Zwart and Donker-Voet (1959).

Etiology. *Listeria monocytogenes* (Murray et al., 1926) Pirie, 1910, is a small Gram-positive rod (Fig. 15.1). Spores are not formed. It is motile, and when grown at 37°C. has a characteristic tumbling movement. This temperature is not so conducive to flagellum production, however, as is room temperature. The organism is a facultative aerobe. On nutrient and liver agar, circular smooth colonies are formed, which are bluish by transmitted light and milk-white by reflected light. After several days' growth on liver

agar the colonies become very viscid. A clear zone of hemolysis is formed around colonies on blood agar. With some strains, hemolysis may not occur on initial isolation but appears after several transfers. Gelatin is not liquefied; indol, acetyl methyl carbinol, and hydrogen sulfide are not formed; and nitrates are not reduced. In a study of the biochemical characteristics of 50 strains of Listeria, Harvey and Faber (1941) found that acid is produced from dextrose, levulose, galactose, maltose, mannose, rhamnose, trehalose, dextrin, and salicin; that lactose, glycerol, sucrose, arabinose, xylose, mannitol, sorbitol, and starch may or may not be fermented; and that acid is not produced from dulcitol, inositol, raffinose, glycogen, and inulin. In the writer's experience, sucrose may not be fermented on first isolation, but after several transfers on artificial culture media, this sugar is usually readily fermented. Dextrose, rhamnose, and salicin are usually fermented rapidly, while the other carbohydrates are usually fermented more slowly. Van Dorssen and Jansen (1951) gave the cultural characteristics of 25 strains isolated in the Netherlands.

Although it may grow quite lightly on initial isolation, after several transplants Listeria grows rather heavily both at 37°C. and at room temperature.

Jones and Woodbine (1961) have reviewed the cultural and other microbiologic characters of *L. monocytogenes*. Füzi and Pillis (1962) differentiated it from Erysipelothrix; Listeria was relatively sensitive to sodium azide and crystal violet, but insensitive to "Mavekal" (disodium hexadecyl disulfonate).

Several serologic studies have been carried out on Listeria. Using the agglutination test, Paterson (1940) recognized four serologic types, while Drew (1916) found two groups with the precipitin test. Jones and Woodbine (1961) reviewed the serologic typing of *L. monocytogenes*. Metzger and Smith (1962) discussed its serologic typing by gel diffusion using thermostable antigens. Njoku-Obi (1962) described an antigen fixation test for serologic diagnosis.

Rudolph and Grässer (1963) used antisera labelled with fluorescent dyes in typing. Sword and Pickett (1961) studied phage typing and developed a system of classification including 8 phage types.

Symptoms. Apparently few clinical symptoms occur in listeriosis in chickens. Paterson (1937) reported that the adult birds died suddenly, while a slow wasting was evident in young naturally affected chickens. The encephalitic symptoms which are characteristic of listeriosis in many mammals have seldom been observed in naturally affected birds, although they have been reported in geese and turkeys.

Mortality in the individual flock may vary within wide limits. Generally only sporadic cases appear, but heavy losses may also occur. Paterson, for example, reported that in one flock 120 out of 200 pullets died over a 3-month period; in another, 191 out of 424 pullets and cockerels died; and in a third flock 8 out of 24 died. In the outbreak described by van der Schaaf and de Jong (1951), 8 of 30 canaries in an aviary died.

Listeriosis has often been found in association with some other disease and may often be secondary to it. Among concurrent diseases and parasites which have been reported are pullorum disease, salmonellosis, fowl pest, lymphomatosis, Newcastle disease, coryza, coccidiosis, heavy tapeworm (*Davainea proglottina* or *Raillietina*) or *Ascaridia galli* infections, heavy mite (*Dermanyssus gallinae*) infestations, ovarian tumor, lowered resistance due to inbreeding, or hatching of imported eggs (Gray, 1958). Stress is apparently an important factor in bringing on attacks of listeriosis.

Observations made so far appear to indicate that young birds are considerably more susceptible to the disease than are adults, although these, too, may die.

Pathology. Listeria ordinarily causes a septicemia in birds. The most striking lesions observed on necropsy of both naturally and experimentally infected chickens are massive necrosis of the heart

FIG. 15.2 — Myocardial necrosis in chickens following intravenous inoculation of Listeria. (Upper left, normal.)

muscle (Fig. 15.2), although this is not an invariable finding. Pericarditis may also be present, and there may be a large amount of fluid in the pericardial cavity.

The most common lesions are necrotic foci in the liver and generalized edema. The liver may be enlarged, congested, and friable, and the spleen may be enlarged and congested. Fibrinous peritonitis and enteritis have also been reported.

The specific name *monocytogenes* was given to Listeria because of the increase in percentage of monocytes in the circulating blood of naturally affected rabbits and guinea pigs. Seastone found that a similar monocytic response is elicited in chickens.

Diagnosis. Listeria septicemia can be diagnosed by isolation of the causative microorganism on artificial culture media. In contrast to the domestic mammals, in which Listeria is often confined to the central nervous system, the organism can be isolated from the abdominal organs

Felsenfeld, O., Volini, I. F., Ishihara, S. J., Bachman, M. C., and Young, V. M.: 1950. A study of the effect of neomycin and other antibiotics on bacteria, viruses and protozoa. Jour. Lab. and Clin. Med. 35:428.

Foley, E. J., Epstein, J. A., and Lee, S. W.: 1944. Effectiveness of penicillin on Listerella. Jour. Bact. 47:110.

Füzi, M., and Pillis, I.: 1962. Die Differenzierung der Listeria monocytogenes und Erysipelothrix rhusiopathiae. Zentr. Bakt., I. Orig. 184:526.

Graham, R., Morrill, C. C., and Levine, N.D.: 1940. Studies on Listerella. IV. Unsuccessful attempts at immunization with living and dead Listerella cultures. Cornell Vet. 30:291.

Gray, M. L.: 1958. Listeriosis in fowls—a review. Avian Dis. 2:296.

———: 1960. Isolation of Listeria monocytogenes from oat silage. Science 132.1767.

———: 1963. Epidemiological aspects of listeriosis. Am. Jour. Pub. Health 53.554.

———, Stafseth, H. J., and Thorp, F., Jr.: 1949. The effect of streptomycin on Listeria. Jour. Am. Vet. Med. Assn. 15:1711.

———, Thorp, F., Jr., and Laine, S. L.: 1950. The effect of aureomycin on Listeria monocytogenes. Bact. Proc. 1950:93.

Harvey, P. C., and Faber, J. E.: 1941. Studies on the Listerella group. I. Biochemical and hemolytic reactions. Jour. Bact. 42:677.

Jasinska, S., Sobiech, T., and Wachnik, Z.: 1962. [Susceptibility of Listeria monocytogenes to antibiotics]. Weterynaria, Wrocław. #12:155. (Abst. Vet. Bull. 33:424.)

Jones, S. M., and Woodbine, M.: 1961. Microbiological aspects of Listeria monocytogenes with special reference to listeriosis in animals. Vet. Rev. Annual 7.30.

Kampelmacher, E. H.: 1962. Listeriose en Volksgezondheid. Tijdschr. Diergeneesk. 87:1664

———, and Jansen, J. M. van N.: 1961. Listeriose bei Mensch und Tier in den Niederlanden von 1956 bis 1960. Wien. Tierärztl. Mschr. 48:442.

Krüger, W.: 1961. Das Vorkommen von Listeria monocytogenes in den verschiedenen Silagen und dessen ätiologische Bedeutung. Arch. Exp. VetMed. 17:181.

Lehnert, C.: 1960. Die Tenazität von Listeria monocytogenes in der Aussenwelt. Zentr. Bakt. I. Orig. 180.350.

Levine, N. D.: 1959. Listeriosis, botulism, erysipelas, and goose influenza. Chap. 15: Diseases of Poultry, 4th ed. Biester, H. E. and Schwarte, L. H. Iowa State Univ. Press, Ames.

Long, P. H., Bliss, E. A., Schoenbach, E. B., Chandler, C. A., and Bryer, M. S.: 1950. The experimental background and clinical use of antibiotics. Lancet 258:1139.

Lucas, A.: 1961. Some aspects of listeriosis in animals in France. Bul. Off. Intern. Epizoot 55.884.

———: 1962. Le diagnostic de la listénose. Bul. Off. Intern. Epizoot 58:97.

———, Laroche, M., Hamel, J., and Chauvrat, J.: 1962. L'infection naturelle à Listeria monocytogenes chez le canard, la perdrix, le faisan, le pigeon. Rec. Méd. Vét. 138:51.

Metzger, J. F., and Smith, C. W.: 1962. Serologic typing of Listeria monocytogenes by gel diffusion using thermostable antigens. Proc. Soc. Exper. Biol. Med. 110.605.

Murray, E. G. D., Webb, R. A., and Swann, M. B. R.: 1926. A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus Bacterium monocytogenes (n. sp). Jour. Path. and Bact. 29 407.

Nilsson, A., and Karlsson, K. A.: 1959. Listeria monocytogenes isolations from animals in Sweden during 1918 to 1957. Nord. Vet. Med. 11:305. (Abst. Vet. Bull. 29 543.)

Njoku-Obi, A. N.: 1962. An antigen-fixation test for the serodiagnosis of Listeria monocytogenes. Cornell Vet. 52:415.

Office International des Epizooties: 1961. La listériose. Bul. Off. Intern. Epizoot. 55:883. (Engl. Trans., p. 937.)

Paterson, J. S.: 1937. Listerella infection in fowls. Vet. Record 49:1533.

———: 1940. The antigenic structure of organisms of the genus Listerella. Jour. Path. and Bact. 51·427

Pirie, J. H. H.: 1940. The genus Listerella Pirie. Science 91:383.

Poznanskaya, L. A.: 1961. [Dietl'not' sohranenija lisleri v ob'ektakh vneshnei sredy]. Veterinariya, Moscow 38(12):21.

———: 1962. Multiplication of Listeria in water. Jour. Microbiol. Epidem. Immunobiol. Moscow 33(9):102. (Abst. Vet. Bul. 33 127)

Pohlmann, E.: 1944. Listerella-Infektionen bei Schafen und Hühnern in Ostpreussen. Deutsch. tierärztl. Wochenschr. u. tierärztl. Rundschau 52/50.13, 127.

Rudolph, W., and Gräser, A.: 1961. Zur Typendifferenzierung von Listeria monocytogenes mit verschiedenfarbig fluoreszierenden Antseren. Monath VetMed. 13 138.

Seastone, C. V.: 1935. Pathogenic organisms of the genus Listerella. Jour. Exper. Med. 62 203.

Seeliger, H. P. R.: 1961. Listeriosis. S. Karger, Basel & New York. x + 308 pp. (English Trans. of the 1958 book published in German)

Sparapani, C.D.: 1946. La bactrellosi dei polli. Clin. Vet., Milano 69.54

Sternberg, H.: 1961 Einige Beobachtungen über die Listeriose in Finnland 1946-1960. Zentr. Bakt. I. Orig. 182·465

Sword, C. P., and Pickett, M. J.: 1961. The isolation and characterization of bacteriophages from Listeria monocytogenes. Jour. Gen. Microbiol. 25:241.

Thamm, H.: 1962. Zur Epizootiologie der Listeriose. Monatsh. VetMed. 17:224.

Train, G.: 1961. Jahresbericht 1960 der Listeriose-Zentralstelle am Staatlichen Veterinärmedizinischen Prüfungsinstitut. Monatsh. VetMed. 16.378.

———: 1962. Jahresbericht 1961 der Listeriose-Zentralstelle am Staatlichen Veterinärmedizinischen Prüfungsinstitut. Monatsh. VetMed. 17:745.

van der Schaaf, A., and de Jong, J.: 1951. Listeriasis (listerellosis) bij een kanarie. Tijdschr. v. Diergeneesk. 76:79.

van der Vlek, J.: 1954. Een geval van encephalitis bij de klp, veroorzaakt door Listeria monocytogenes. Tijdschr. v. Diergeneesk. 79.972.

van Dorssen, C. A., and Jansen, J.: 1951. Over het practische belang van Listeria-infectie. Tijdschr. v. Diergeneesk. 76:756.

Welshimer, H. J.: 1960. Survival of Listeria monocytogenes in soil. Jour. Bact. 80:316.

Zink, A., de Mello, G. G., and Burkhart, R. L.: 1951. Listenosis—field and laboratory studies, and aureomycin activity. Am. Jour. Vet. Res. 12:194.

Zwart, P., and Donker-Voet, J.: 1959. Listeriosis bij in gevangenschap gehouden dieren. Tijdschr. Diergeneesk. 84:712

Botulism

Botulism (limberneck, *bulbar paralysis*) is a disease which may affect not only chickens and other domestic birds, but also man and the domestic mammals. It is a type of food poisoning which results from ingestion of spoiled foods in which the bacterium, *Clostridium botulinum*, has been growing and producing toxins. Other bacteria which cause food poisoning in man, such as staphylococci, have not been recognized as a cause of the same condition in domestic animals.

The earliest report of botulism as the cause of so called limberneck in chickens was made in the United States by Dickson (1917, 1918). Spoiled canned corn, green beans, and apricots were found to have caused outbreaks in four flocks; in three of these cases, humans also died as a result of eating the same food. Hart (1920), Wilkins and Dutcher (1920), Graham and Schwarze (1921), Doyle (1923), Graham and Boughton (1923), and McLachlan and Newton (1952) also reported outbreaks of botulism in chickens. Van Heelsbergen (1929) observed symptoms of limberneck in Holland. The disease also occurs in other species of birds. Among others, Graham and Boughton (1923), Theiler (1927), Dadot (1945), Szyfres et al. (1948), and Gunning (1950) reported it in ducks; Martinaglia (1937) and Gunning (1950) in geese; Coburn and Quortrup (1938a) in turkeys; Palmer and Baker (1922), and Dobberstein

and Piening (1933) in swans; Dinter and Kull (1954), Vadlamudi et al. (1959), and Lee et al. (1962) in game farm pheasants, and Theiler (1927) in the ostrich. It has also been described in numerous species of wild waterfowl, in which it is an important cause of death. An interesting exception reported by Kalmbach (1939) is the vulture. This bird, which lives on decaying carcasses, is tremendously resistant to *Clostridium botulinum* toxins, withstanding as much as 300,000 guinea pig minimum lethal doses. This is a tolerance to 0.04 ml. toxin injected per gram of body weight.

Etiology. Botulism is caused by the toxins which are a metabolic product of the growth of *Clostridium botulinum*. Some fifteen subtypes of this species have been described by Meyer and Gunnison (1929a, b, c, d) on the basis of toxicity, agglutination, and fermentation reactions. Of these, *Clostridium botulinum* Type A and *Cl. botulinum* Type C are the most common causes of botulism in domestic birds. Hazen (1942) reported that a strain of *Cl. botulinum* Type E isolated from canned sprats was not toxic for chickens, but that a strain of the same type isolated from smoked salmon was.

Clostridium botulinum is an anaerobic, Gram-positive, sporeforming, large rod. It is widely dispersed in the soil and enters food as a contaminant. The mere presence of the organism, however, is in-

sufficient to cause disease or to be of diagnostic significance. Only when the organism has grown, multiplied, and produced toxins does the food become dangerous. Since growth requires anaerobic conditions, canned foods furnish an excellent culture medium. Their improper sterilization at the time of canning may result in spoilage due to *Clostridium botulinum* or other organisms. It has been noted that certain foods are less liable to spoilage than others. Those which are acid, such as cherries and other acid fruits, are unfavorable for the growth of Clostridium, while canned vegetables, such as corn and green beans, are very favorable. Hence more cases of botulism are associated with the latter type of food.

Botulism can result from consumption of carcasses of birds which have died of the disease, and also of the maggots of the blue-bottle fly, *Lucilia caesar*, from spoiled meat. The toxin formed in the meat by Clostridium is ingested by the maggots, rendering them extremely poisonous. Wilkins and Dutcher (1920) reported the toxic character of *Lucilia* larvae, but were unable to produce limberneck in chickens with the larvae of *Calliphora vomitoria* or *Musca domestica*. In an outbreak of botulism on a pheasant farm in which 4,000 birds died in two weeks, Lee et al. (1962) found *Cl. botulinum* Type C toxin in *Lucilia illustris* larvae from pheasant carcasses and feed wastes and also in adult flies. One gram of larvae contained 180,000 mouse LD_{50}'s, and pheasants died after eating eight or more third instar larvae.

A number of carbohydrates are fermented with the production of acid and gas. Different strains and types may vary in the specific carbohydrates which they attack. In milk, Type A produces slight acidity and a slow curdling precipitate, followed by digestion and darkening. Type C produces a slowly increasing acidity, without coagulation or digestion. Type A blackens and digests meat and brain media, causing an odor of putrefaction, while Type C does not. Both types liquefy gelatin, but Type A also

liquefies coagulated albumin, while Type C does not. Growth is in the form of compact, rather lenticular discs, which may later become fluffy.

Symptoms. Outbreaks of botulism are usually traceable to the feeding of spoiled canned food, decomposed meat or vegetables, or spoiled grain. Feed contaminated by feces of affected birds, the bodies of birds which have died of the disease, or the maggots of certain flies living in such carcasses may contain enough toxin to cause the disease.

Symptoms may appear within a few hours to a day or two after the spoiled food is eaten. The most common symptom is paralysis. The legs and wing muscles are usually the first affected. The birds become unable to walk, and their wings rest on the ground. If the neck muscles are affected, the head hangs limp. This symptom is responsible for the name "limberneck." In the early stages of botulism in poultry, the eyes are dull and partly closed. The chickens are inactive and show symptoms of weakness and unsteadiness when they move. The feathers are ruffled, and the birds refuse to eat.

In mild cases the leg weakness and drowsiness may disappear, and the affected birds recover in 2 or 3 days. In

FIG. 15.3 — Chicken affected with botulism.

severe cases, however, death may occur in a few hours. Fatally affected birds lie in a profound coma, appearing lifeless, for several hours before death (Fig. 15.3). In the advanced stages a broken quivering of the feathers may be observed, and in some cases large numbers of feathers are shed. Looseness of the feathers is often seen in botulism. Soft, pasty feces or even diarrhea may be observed in some cases. The severity of the symptoms and the prognosis depend on the amount of toxin ingested.

Gross pathology. Few lesions are seen at necropsy. A slight catarrhal enteritis may be present, or small but intense inflamed hemorrhagic areas may sometimes be observed. Portions of the intestine may be dilated or distended. Other gross changes are usually lacking.

Pathogenesis. As stated above, all birds which have ingested C. botulinum toxin do not die of the intoxication. The prognosis depends primarily on the amount which has been eaten. However, this toxin is one of the most powerful poisons known. Bengston (1924) found that the minimum lethal dose for guinea pigs was 0.00012 mg. per kg. when administered subcutaneously. This may be compared with the minimum lethal dose of cobra venom under similar conditions, 0.002 mg. per kg., and with that of the alkaloid aconitine, 0.06 mg. per kg.

Although it is relatively heat-stable, botulinum toxin may be destroyed by sufficient boiling. The concentration of toxin and perhaps other factors may, however, markedly affect the time necessary for its destruction. For instance, Schoenholz and Meyer (1924) found that botulinum toxin was destroyed in 6 minutes at 80° C., but that the toxin in spoiled vegetables was much more resistant due to its high concentration and to slow heat penetration.

Western duck sickness. For many years a disease of wild ducks and other water birds has been known to occur in the western part of the United States. It was studied by Wetmore (1918), who concluded that it was due to alkali poison-

ing. More recently, however, it was shown by Kalmbach (1930) and Giltner and Couch (1930) that western duck sickness is botulism.

This disease is responsible for a tremendous number of deaths among wild waterfowl. Indeed, Kalmbach (1935a, b) considers it one of the most important causes of mortality among these birds. It has been estimated that a million birds died at Malheur Lake in eastern Oregon in 1925, one to three million at Great Salt Lake in 1929, and a quarter of a million at the northern end of Great Salt Lake in 1932. In 1910 the greatest recorded epidemic occurred in the western states, causing the death of hundreds of thousands of birds. Kalmbach (1935a) states that in recent years the epidemics have increased in frequency and that the range of the disease has also increased. Kalmbach and Gunderson (1934) list outbreaks as having occurred in Alberta and Saskatchewan in Canada; Oregon, California, Nevada, Arizona, New Mexico, Texas, Utah, Idaho, Montana, Kansas, Nebraska, North Dakota, South Dakota, and Minnesota in the United States; and Mexico. Reference should be made to their paper for a comprehensive discussion of the disease. It has since been reported in Australia by Pullar (1933, 1934), Rose (1934), and Farleigh (1949); in Alberta, Canada, by Shaw and Simpson (1936); and at Tulare Lake, California, by McLean (1946). It has also been studied by Gunnison and Coleman (1932) and Coburn and Quortrup (1938b). An interesting account of the disease together with the measures which are being taken to combat it at Tule and Lower Klamath Lakes in California is given by McLeod (1950).

According to Kalmbach and Gunderson, 69 species of birds belonging to 21 families are known to have been affected with western duck sickness. Among these are herons, geese, ducks, hawks, sandpipers, gulls, and blackbirds. Domestic birds may also die if given access to areas in which the disease is present.

Western duck sickness is caused by *Clostridium botulinum* Type C. This organism and its toxins have been isolated from the livers of affected birds, mud, decaying vegetation, various fly larvae, and from dead fish, dead grasshoppers, and grain lying in the water. Outbreaks are associated with a number of factors, among them being prevalence of organic matter, a slight alkalinity (pH 7.5 to 9) which favors growth and toxin production, and a high temperature (37° C. optimum). The incidence of the disease is closely correlated with shallow stagnant water and mud flats. Quortrup and Holt (1941) showed that anaerobic conditions favorable for the growth of the organism are produced by decaying vegetable matter, such as aquatic plants killed by evaporation or terrestrial plants killed by flooding. They reported that potential toxin-producing areas could be detected by determining the pH and oxygen content of the water.

The possibility that *Pseudomonas aeruginosa* might be a factor in the production of botulinus toxin in these areas was suggested by Quortrup and Sudheimer (1943a). They isolated this organism frequently from the water of duck marshes and from wild duck intestines. *Ps. aeruginosa* utilizes oxygen and produces an alkaline medium, and these investigators showed that when it is grown together with *Cl. botulinum*, an increased amount of botulinus toxin is produced.

Diagnosis. Diagnosis of botulism in domestic birds can be made on the basis of the paralytic symptoms, loose feathers, usual absence of marked gross lesions, and history of eating spoiled food, decaying carcasses, etc. Paralysis of the nictitating membrane of the eye occurs frequently, although it is not pathognomonic. Demonstration of the toxins in the digestive tract by feeding or inoculation of experimental animals may also be resorted to. According to Quortrup and Sudheimer (1943b) the toxin is present in such an amount in the blood of affected wild ducks that it can be detected by inoculating mice in-

traperitoneally with 1 ml. of serum. Mice immunized against the toxin fail to show symptoms, while nonimmunized mice may die. The organism itself can be recovered by the use of anaerobic culture media. However, it must be borne in mind that isolation of *Clostridium botulinum* from the intestinal tract does not necessarily predicate a diagnosis of botulism, since this bacterium is commonly found in soil and may be isolated from the alimentary canal of healthy, normal birds or mammals.

Among common media utilized in the cultivation of Clostridium are meat mash, brain mash, deep tubes of liver agar, and thioglycollate broth. All these media should be brought to the boiling point and allowed to cool immediately before inoculation.

Therapy and prophylaxis. Prevention of botulism in poultry depends primarily on feeding a wholesome ration. The feeding to chickens of spoiled canned foods, tainted meats, or decomposed vegetables should be avoided, and the danger to fowls of putrefying carcasses should be recognized.

The bodies of chickens which have died of any disease are a source of danger and should be destroyed by burning. Since the droppings of affected birds contain toxin, sick birds should be isolated and their droppings removed to fields that cannot be reached by poultry.

Laxatives such as castor oil or Epsom salt are of value in the treatment of exposed birds which have not yet shown symptoms of the disease. These agents can be mixed with bran in the form of a wet mash. Mildly affected chickens which cannot eat may be dosed individually with one-half ounce of castor oil. One pound of Epsom salt for each 75 to 100 chickens may be mixed in the feed for flock treatment. All other feed should be withheld, and the treatment repeated until the digestive tract is empty. The recovery of affected birds will be aided if they are kept in a cool, shady place.

Injection of antitoxin is of value in

human botulism, and Brandly (1957) found that an aluminum hydroxide-adsorbed botulinus antitoxin gave good protection to pheasants against botulism. Boroff and Reilly (1959) immunized pheasants and ducks with two injections 3 or 4 weeks apart of a mixture of *Cl. botulinum* Type C toxoid and Freund's incomplete adjuvant. Quortrup and Sudheimer (1942) reported that the majority of birds affected with western duck sickness would recover if treated with Type C botulinus antitoxin. At the Tule Lake Refuge, according to McLeod (1950), thousands of wild ducks were treated intraperitoneally with antitoxin, large ducks receiving 4 ml. and small ones 2 ml. Seventy per cent of the

treated birds recovered. The average cost of antitoxin was estimated to be 11 cents a duck. However, Rosen and Bischoff (1953) stated that such duck rescue work costs three to five dollars per bird rescued. They used a different and much more successful approach during an outbreak of botulism in the Tulare Lake Basin in 1952. By herding the ducks away from dangerous areas by plane, air-thrust boat, and through the use of pyrotechnics, by pumping operations carried out by the area farmers, and by distributing feed in safe areas, they reduced the mortality among 2 million ducks to 1 per cent as compared with an expected mortality of 20 per cent.

REFERENCES

Bengston, I. A.: 1921. Studies on organisms concerned as causative factors in botulism. U.S. Pub. Health Serv. Hyg. Lab. Bul. 136.

Boroff, D. A., and Reilly, J. R.: 1959. Studies of the toxin of *Clostridium botulinum*. V. Prophylactic immunization of pheasants and ducks against avian botulism. Jour. Bact. 77:142.

Brandly, C. A.: 1957. Personal communication.

Coburn, D. R., and Quortrup, E. R.: 1938a. Atypical botulism in turkeys. Jour. Am. Vet. Med. Assn. 93:485.

——, and Quortrup, E. R.: 1938b. The distribution of botulinus toxin in duck sickness areas. Trans. Third No. Am. Wildlife Conf. P. 869.

Dadd, F.: 1915. Botulism the canards. Rec. Méd. Vét. 121:177.

Dickson, E. C.: 1917. Botulism. A cause of limber-neck in chickens. Jour. Am. Vet. Med. Assn. 50:612.

——: 1918. Botulism. A clinical and experimental study. The Rockefeller Inst. Med. Res. Monograph No. 8.

Dinter, Z., and Kull, K. E.: 1954. Über einem Ausbruch des Botulismus bei Fasanenküken. Nordisk Veterinärmed. 6,866

Dobberstein, J., and Piening, C.: 1933. Beiträge zur Pathologie des Zentralnervensystems bei Tieren. I. Botulismus bei Schwänen. Berliner tierärztl. Wochenschr. 49.549.

Doyle, L. P.: 1923. Limberneck in chickens. Jour. Am. Vet. Med. Assn. 63:754.

Faileych, E. A.: 1919. Botulism in wild fowl. Yearbook Inst. Insp. Stock, New So. Wales, 1949.101.

Gilner, L. T., and Couch, J. F.: 1950. Western duck sickness and botulism. Science 72.660.

Graham, R., and Boughton, I. B.: 1923. *Clostridium botulinum* Type C. A pathogenic anaerobe associated with a limberneck-like disease in chickens and ducks. Ill. Agr. Exper. Sta., Bul. 246.

——, and Schwarze, H.: 1921. Avian botulism (Type A) or limber neck. Jour. Infect. Dis. 28 317.

Gunning, O. V.: 1950. Losses in geese, ducks and poultry, caused by a toxin in the gut contents which resembled the toxin produced by the anaerobe *Cl. botulinum*. Brit. Vet. Jour. 106.81.

Gunnison, J. B., and Coleman, G. E.: 1932. *Clostridium botulinum* Type C associated with western duck disease. Jour. Infect. Dis. 51:542.

Hart, G. H.: 1920. Clinical and case reports. Botulism in chickens. Jour. Am. Vet. Med. Assn. 57:75

Hazen, E. L.: 1942. Differential characters of two strains of *Clostridium botulinum* Type E: Action of toxins in chickens. Proc. Soc. Exper. Biol. and Med. 50.112.

Kalmbach, E. R.: 1930. Western duck sickness produced experimentally. Science 72.658.

——: 1933a. Will botulism become a world-wide hazard to wild fowl? Jour. Am. Vet. Med. Assn. 87 183.

——: 1935b. Botulism is a factor in the decrease of western waterfowl. U.S.D.A. Yearbook. P. 140.

——: 1939. American vultures and the toxin of *Clostridium botulinum*. Jour. Am. Vet. Med. Assn. 94.187.

——, and Gunderson, M. F.: 1934. Western duck sickness: A form of botulism. U.S.D.A. Tech. Bul. 411.

Lee, V. H., Vadlamudi, S., and Hanson, R. P.: 1962. Blow fly larvae as a source of botulinum toxin for game farm pheasants. Jour. Wildl. Mgmt 26:411.

McLachlan, J. J., and Newton, L. G.: 1952. Botulism in poultry Queensland Agr. Jour. 74:87.

McLean, D. D.: 1946. Duck disease at Tulare Lake. Calif. Fish and Game 32:71.

McLeod, E. R.: 1950. Duck botulism. Sci. Monthly 71:502.

Martinaglia, G.: 1937. Some considerations regarding the health of wild animals in captivity. So. African Jour. Sci. 33.333.

Meyer, K. F., and Gunnison, J. B.: 1929a. European strains of Clostridium botulinum. XXXVI. Jour. Infect. Dis. 45:96.

——, and Gunnison, J. B.: 1929b. South African cultures of Clostridium botulinum and parabotulinum. XXXVII. Jour. Infect. Dis. 45:106.

——, and Gunnison, J. B.: 1929c. Cultural study of an international collection of Clostridium botulinum and parabotulinum. XXXVIII. Jour. Infect. Dis. 45:119.

——, and Gunnison, J. B.: 1929d. Botulism due to home canned Bartlett pears. XXXIX. Jour. Infect. Dis. 45:135.

Palmer, C. C., and Baker, H. R.: 1922. Botulism (Limber Neck) in swans. Ohio State Univ., Vet. Alumni Quart. 10.93.

Piening, C.: 1953. Botulismus bei Schwanen. Tierarztl Rundschau 39 120

Pullar, E. M.: 1933. Limberneck (botulism) in ducks. Australian Vet. Jour. 9.26.

——: 1934. Enzootic botulism amongst wild birds. Australian Vet. Jour. 10:128.

Quortrup, E. R., and Holt, A. L.: 1941. Detection of potential botulinus toxin-producing areas in western duck marshes with suggestions for control. Jour. Bact. 41.363.

——, and Sudheimer, R. L.: 1942. Research notes on botulism in western marsh areas with recommendations for control. Trans. Seventh No. Am. Wildlife Conf. P. 284.

——, and Sudheimer, R. L.: 1943a. Some ecological relations of Pseudomonas aeruginosa to Clostridium botulinum Type C. Jour. Bact. 45:551.

——, and Sudheimer, R. L.: 1943b. Detection of botulinus toxin in the blood stream of wild ducks. Jour. Am. Vet. Med. Assn. 102:264

Rose, A. L.: 1934. Enzootic botulism amongst wild birds Australian Vet. Jour. 10:175

Rosen, M. N., and Bischoff, A. L.: 1953. A new approach toward botulism control. Proc. 18th No. Am. Wildlife Conf. 1953:191.

Schoenholz, P., and Meyer, K. F.: 1924. Effect of direct sunlight, diffused daylight, and heat on potency of botulinus toxin in culture mediums and vegetable products. XXIV. Jour. Infect. Dis. 35:361.

Shaw, R. M. and Simpson, G. S.: 1936. Clostridium botulinum Type C in relation to duck sickness in the Province of Alberta. Jour. Bact. 32:79.

Snyfres, B., Trenchi, H., and Abrarzon, D.: 1948. Intoxication botulinique des canards. Bul. Off. Intern. Epizoot. 29:431.

Theiler, Ar.: 1927. Lamsiekte (parabotulism) in cattle in South Africa. Union So. Afr. Dept. Agr. Rep. 11-12, Div. Vet. Ed. and Res., pt. 2 p421.

Vadlamudi, S., Lee, V. H., and Hanson, R. P.: 1959. Case report — botulism type C outbreak on a pheasant game farm. Avian Dis. 3.344.

van Heelsbergen, T.: 1929. Handbuch der Gefiugelkrankheiten und der Gefiugelzucht. Ferdinand Enke, Stuttgart.

Wetmore, Ar.: 1918. The duck sickness in Utah. U.S.D.A., Bul. 672.

Wilkins, S. D., and Dutcher, R. A.: 1920. Limberneck in poultry. Jour. Am. Vet. Med. Assn. 57.655.

Erysipelas
(Gefluegelrotlauf)

A septicemia associated with *Erysipelothrix insidiosa*, the causative agent of swine erysipelas, has been reported in many species of birds. Jarosch (1905) was the first to recognize this disease in birds, isolating the organism from a turkey. Subsequently the disease has been found to occur also in the chicken, duck, pigeon, pheasant, quail, peacock, and a number of wild birds. It is found throughout the world. Since Jarosch's initial paper, erysipelas has been described in turkeys by

Eber (1921), Beaudette and Hudson (1936), Madsen (1937), Hoffman and Hinshaw (1938), Van Roekel et al. (1938), Rosenwald and Dickinson (1939, 1941), Rosenwald (1940), Schlotthauer and Thompson (1940), Lindenmayer and Hamilton (1942), Burnett and Hofstad (1943), Lindenmayer (1943), Jungherr and Gifford (1944), Stiles (1946), Moore (1947), Brown et al. (1949), Blaxland et al. (1949), Bivins (1949), Hudson (1949), DeLay and Koch (1950), Peterson and

Hymas (1950), Dunne et al. (1951), Moore (1951), Byrne et al. (1952), Hudson et al. (1952), Bivins et al. (1955), Elliott (1956), and Raines and Winkel (1956).

Hausser (1909) first described the disease in the chicken. It has since been reported in this bird by Schipp (1910), Broll (1911), Pfaff (1921), Reinhardt (1924), Scholl and Jacquart (1926), van Heelsbergen (1929), Schmidt-Hoensdorf (1931), Sparapani (1938), Breed (1943), Evans and Narotsky (1954), Juslin and Stenberg (1954), Bivins et al. (1955), and Hall (1963). Poels (1919) first encountered erysipelas septicemia in ducks, and this finding was confirmed by Eber (1921), Scholl and Jacquart (1926), Werner (1932), Horstmann (1938), Graham et al. (1939), White and Henley (1942), Doria (1943), Hudson (1949), Hudson et al. (1952), Zeiger (1952), Galli (1953), Dougherty and Bruner (1954), Engel and van der Maas (1955), Valentin et al. (1956–57), Murase et al. (1959), and Nikolić and Sepic (1961).

It was reported in a wild mallard (Anas pl. platyrhynchos) by Bourgeois (1944). The disease was recognized in the pigeon by Poels (1919) and de Mendonça Machado (1945); in the quail by Jármai (1920), Waller (1939), and van Dorssen and Donkervoet (1953); in the pheasant by Vianello (1898), Morgan (according to Waller, 1939), Szabó (1943), Hudson et al. (1952), Rossi and Pini (1953), Raines and Winkel (1956), Valentin et al. (1956–57), and Trbić and Tadić (1959); in the peacock by Greener (1959); in the goose by Linsert (1914); in the green finch by de la Villa (1934); in the ring-necked parakeet (Palaeornis torquata) by Urbain et al. (1945); in the white stork (Ciconia ciconia) and herring gull (Larus argentatus) by Christiansen (1919); and in birds in zoological gardens by Jármai (1920) and Schmidt-Hoensdorf (1931). Van Bemmel et al. (1960) found it in fruit pigeons (Ducula sp.) and fisheating birds such as the crowned crane (Balearica pavonina) and Porphyrio sp. in the Rotterdam zoo.

The same organism, or a very similar one, has also been found in the sheep, ox,

horse, dog, mink, guinea pig, mouse, rat, chipmunk, porpoise, several species of fish, and man (Van Es and McGrath, 1936; Stiles, 1944; Drake and Hall, 1947; Grey, 1947a; Connell, 1954; Seibold and Neal, 1956).

Etiology. The etiologic agent, *Erysipelothrix insidiosa* (Syn., *E. rhusiopathiae*), can be isolated culturally from many of the organs of affected birds. Since the disease is a septicemia, the heart blood, liver, pericardial fluid, and pathologic accumulations of fluid usually contain large numbers of the organisms. The bacterium has also been isolated from diphtheritic membranes on the pharyngeal and nasal mucosae. While isolation may be accomplished on plain nutrient agar, it is more uniformly successful if blood or serum is added to the medium.

E. insidiosa on first isolation is a Gram-positive rod, but after continued transfer on artificial culture media it may become Gram-negative. In the animal body and when first isolated, the organisms may be relatively short ($1-2\mu$ long), but on cultivation they ordinarily are in the form of slender filaments $4-15\mu$ long (Fig. 15.4). Branching of the filaments has been described. The organism forms no spores and is nonmotile. The colonies are round and translucent, usually about 1 mm. in diameter. On first isolation they

FIG. 15.4—*Erysipelothrix insidiosa*, agar culture. X2,000. (From Nowak: Documenta Microbiologica, courtesy Gustav Fischer.)

are smooth, but, on continued transfer, larger, rough colonies may appear. Gelatin is not liquefied, but a typical "test tube brush" growth occurs along the line of the stab. Litmus milk may be slightly acidified. A narrow zone of green hemolysis forms on blood agar. Neither indol nor acetyl methyl carbinol is formed, but hydrogen sulfide is produced. Gas is not formed from carbohydrate media. According to Karlson (1938), acid is formed in dextrose, lactose, galactose, and levulose, while mannose and cellobiose are fermented late, and no acid is formed from arabinose, xylose, rhamnose, maltose, melibiose, sucrose, trehalose, raffinose, melezitose, dextrin, starch, inulin, amygdalin, salicin, glycerol, erythritol, adonitol, mannitol, sorbitol, dulcitol, or inositol. Maximum growth occurs at a pH of 7.6 at 37.5° C. Füzi and Pillis (1962) differentiated between *E. insidiosa* and *Listeria monocytogenes*; the former is relatively resistant to crystal violet and sodium azide, but is relatively sensitive to "Mavekal" (disodium hexadecyl disulfonate) *in vitro*. Further information on the bacteriology of the organism is given by Woodbine (1950), Byrne *et al.* (1952), and Juslin and Stenberg (1954).

Symptoms and pathology. *Turkey.* Erysipelas usually affects poults from four to seven months of age, although Rosenwald (1940) reported it in a 7-day-old poult. It usually appears in the fall, most outbreaks which have been reported having occurred in September, October, and November. Losses may be fairly heavy, mortalities of from 2½ to 25 per cent having been reported in different flocks. An interesting characteristic of this disease is the fact that male birds are apparently much more susceptible than females. This differential sex incidence has been reported by a number of writers. Madsen (1937), for instance, stated that out of 1,200, 325 turkeys which died in a flock of 1,200, only 25 were females, while Rosenwald and Dickinson (1941) reported that 82.1 per cent of the turkeys affected in outbreaks in 16 flocks were males.

Symptoms include general weakness and listlessness, inappetence, and sometimes a yellowish or greenish diarrhea. Affected birds stand or crouch with lowered head, drooping wings and tail, and ruffled feathers. The skin and wattles may be cyanotic. Dyspnea and an ulcerated nasal mucosa may be present. Rosenwald and Dickinson (1941) consider the most pathognomonic lesion to be a turgid, reddish-purple caruncle.

At necropsy the most characteristic lesions are petechial and diffuse hemorrhages in many of the tissues and organs. They have been reported in the abdominal, pectoral, and femoral muscles, fascia, peritracheal tissues, pleura, peritoneum, pericardium, heart, lungs, spleen, and small intestine. The liver is enlarged, congested, and often friable or mottled. In some cases necrotic foci may be present in this organ. The spleen is usually enlarged, congested, and friable, and hemorrhages or necrotic foci may be observed. The kidneys may be enlarged and congested. The lungs may be congested or brownish in color. A catarrhal exudate which may be sanguineous is present in the intestine, and the intestinal mucosa may be inflamed, edematous, hemorrhagic, or even necrotic. The mesenteric and other blood vessels are often engorged. A nasopharyngeal catarrh has been observed in some cases. Inflammation of the mucosa of the proventriculus has been reported, and a swollen joint was noted in one case by Beaudette and Hudson (1936). On the other hand, in the cases in week-old poults reported by Rosenwald (1940), characteristic lesions were absent on necropsy.

Peterson and Hymas (1950) reported a chronic cutaneous form of the disease in a single male breeder turkey from a Washington state flock. The breast was covered by a large, thick, leathery brown wheal, and similar lesions were present in the skin on other parts of the body. Erysipelothrix was isolated from the necrotic tissue, but not from the internal organs, which appeared normal. For further in-

formation on the disease in turkeys, see the discussion in Chapter 11 of this book.

Chicken. Symptoms of lassitude, inappetence, and diarrhea may be present in the chicken. The pathological anatomical changes induced in this bird by *Erysipelothrix insidiosa* are similar to those in the turkey, but are usually not so marked. The report by Sparapani (1938) of a paralysis of chickens due to lumbar meningitis caused by *Erysipelothrix insidiosa* is of interest.

Duck. Ducklings are susceptible to erysipelas. Losses may be quite high. In one flock of 46,000, Graham *et al.* (1939) reported a loss of almost 25 per cent. In the duck the gross pathologic lesions are similar to those in the turkey and chicken. A serofibrinous exudate may be present in the air sacs, and the lungs are often congested. The liver is often enlarged, friable, mottled, and may contain numerous yellowish pin-point foci. The spleen is usually congested, enlarged, and soft. Petechial hemorrhages may be present in the heart. Areas of congestion are often found in the intestine, and catarrhal enteritis may be observed. Dark, congested areas in the webs of the feet, and chronic enlargement of the femorotibial articulations have also been reported. Congestion may be found in many of the organs, and petechiation of the muscles may also occur.

The symptoms and lesions caused by *Erysipelothrix insidiosa* in other species of birds are similar to those described above.

Pathogenesis. The pathogenicity of *Erysipelothrix insidiosa* for pigeons is well established. Indeed, in some laboratories, inoculation of these birds is a routine procedure for the diagnosis of swine erysipelas. While the turkey is quite susceptible to the disease, its susceptibility is increased by other infectious diseases, malnutrition, or any condition which lowers the bird's vitality. Among predisposing factors are confinement, overcrowding, damp or inclement weather, drafts, sudden temperature changes, and poor sanitation. Of interest in this connection is the report of Marinelli (1928)

that the feeding of polished rice lowers the resistance of pigeons to Erysipelothrix. It is noteworthy that most of the outbreaks of erysipelas reported have occurred in the fall and winter months.

While the pathogenicity of *E. insidiosa* is about the same for the duck as for the turkey, it is definitely lower for the chicken. Most of the cases in this species have been sporadic rather than epidemic in nature, and other diseases such as pasteurellosis, tuberculosis, and leukosis have often been present also. Experimental inoculations of the organism by a number of workers give further confirmation of the relatively greater resistance to infection of the chicken.

Diagnosis. While extensive petechiation is quite characteristic of erysipelas in birds, reliance cannot be placed upon this lesion alone in arriving at a diagnosis. A positive diagnosis can be made only by isolating the causative microorganism from the tissues of affected birds by cultural methods, and identifying it. For best results, cultures of heart blood, liver, or other organs should be seeded on blood or serum agar.

A presumptive diagnosis can often be made by examination of thin blood smears stained by Gram's method. The organisms may be abundant in the heart blood or spleen. Filamentous forms are usually absent in the animal tissues.

A serological test might be helpful, since it has been reported by a number of workers that high agglutinin titers may result from natural or experimental infection.

Epidemiology. Erysipelothrix is generally considered able to live for a long time in the soil, so that infected premises are a source of infection. However, Doyle (1960) reviewed the evidence for this belief and concluded that there is no evidence that *E. insidiosa* can multiply in the soil, that the length of time it can survive in the soil is no greater than that of many other non-sporeforming pathogenic bacteria, and that the soil is therefore not the most important source of infection. In this connec-

tion, Gurova (1959) found that *E. insidiosa* would survive only 21 to 30 days in damp, nonsterilized, Ukrainian black-earth type soil in petri dishes, although it survived much longer in sterilized soil.

E. insidiosa has been recovered from fish meal (Grenci, 1943), so mixed feeds may also be a source. However, Kubiš (1942) reported that the organism was killed in the small intestine after ingestion by the pigeon. Infected swine, sheep, rats, and fish are reservoirs of infection for birds.

It has been suggested that the organism enters the body through scratches or abrasions in the skin. In addition, Wellmann (1950) found that *E. insidiosa* could be transmitted mechanically from sick mice to pigeons by the stable fly, horsefly, mosquitoes, and other biting flies.

Erysipelothrix is infectious for man; infections in persons who have handled or performed necropsies on sick birds have been reported by Bivins (1949), Narotsky and Hawley (1949), Dunne *et al.* (1951), Hudson *et al.* (1952), and Evans and Narotsky (1954).

Therapy. Penicillin, and particularly its longer-lasting derivatives such as procaine penicillin or benzathine penicillin, have been used with success in treating affected turkeys, pigeons, and pheasants, usually by intramuscular injection (Heilman and Herrell, 1944; Van Es *et al.*, 1945; Gifford and Jungherr, 1946; Grey, 1947a, 1947b; Brown *et al.*, 1949; Prier and Alberts, 1950; DeLay and Koch, 1950; Dunne *et al.*, 1951; Moynihan and Stovell, 1954; Smith, 1956; Jerstad and Johns, 1956; Raines and Winkel, 1956). In addition, Elliott (1956) and Boyer and Brown (1957) reported that administration of procaine penicillin in the feed was effective in controlling the outbreaks of erysipelas in turkeys. Boyer and Brown (1957) used 800 gm. per ton of feed, while Elliott (1956) used 200 gm. per ton the first week, 120 gm. per ton the second week, and 64 gm. per ton the third week. However, Raines and Winkel (1956) reported an outbreak in turkeys due to a penicillin-resistant strain

of Erysipelothrix which was sensitive to dihydrostreptomycin.

The tetracycline group antibiotics are also quite active (Prier and Alberts, 1950; Moynihan and Stovell, 1954; Smith, 1956). Boyer and Brown (1957) found that 325–600 gm. of chlortetracycline per ton of feed reduced mortality in affected turkeys. Orlandella (1955) cured experimentally infected pigeons with erythromycin administered either orally or subcutaneously.

Streptomycin is much less effective than penicillin (Woodbine with Cheeseman, 1947; Grey, 1947c), but Dunne *et al.* (1951) found that dihydrostreptomycin was effective in treating turkeys, and Raines and Winkel (1956) used it successfully against a penicillin-resistant strain of Erysipelothrix.

The sulfonamides are apparently valueless for treating *E. insidiosa* infections (Konst, 1945; Frei and Jezierski, 1945; Woodbine, 1946, 1950) as is furazolidone (Smith, 1956; Jerstad and Johns, 1957). Further information on the chemotherapy of Erysipelothrix is given by Woodbine (1950), Moynihan and Stovell (1954), and Smith (1956).

Immunization. A formalin-killed, aluminum hydroxide-adsorbed *E. insidiosa* bacterin has been used successfully by a number of workers to immunize turkeys (Adler and Spencer, 1952; Dickinson *et al.*, 1953; Jerstad and Johns, 1954a, 1954b; Boyer and Brown, 1956; Cooper *et al.*, 1954, 1957; Boyer and Brown, 1957). This immunity is not great enough to protect all birds against experimental challenge, but it has been found of practical value under field conditions. It can be used in conjunction with penicillin treatment of sick birds at the beginning of an outbreak to control losses. Mitrovic *et al.* (1961) found that a concentrated, formolized, adsorbed vaccine made from variant smooth strains H4 and H7 of *E. insidiosa* protected turkeys better than a commercial vaccine made from five rough strains.

In a preliminary study, Osebold *et al.* (1950) found that vaccination with a live, avirulent culture of *E. insidiosa* pro-

tected turkeys against challenge with a virulent strain 12 weeks later. Peterson and Hymas (1952) immunized turkeys by simultaneous injection of a live, attenuated culture and antiserum.

Treatment with antiserum alone may be of some value, but only if it is administered very early or before clinical symptoms appear. Sparapani (1938) reported the successful use of swine erysipelas antiserum in chickens, as did White and Henley (1942) in ducks, Breed (1943) in chickens, and Grey (1947b) in turkeys, if

it was given early enough. On the other hand, attempts by Graham et al. (1939) to protect ducklings on contaminated premises by the use of antisera and bacterins were unsuccessful. Brown et al. (1949) gave antiserum to an infected flock of turkeys with equivocal results. Rosenwald and Dickinson (1941) reported that the use of commercial swine erysipelas antiserum was impractical in the treatment or prevention of the disease in turkeys.

REFERENCES

Adler, H. E., and Spencer, G. R.: 1952. Immunization of turkeys and pigs with an erysipelas bacterin. Cornell Vet. 42 233

Beaudette, F. R., and Hudson, C. B.: 1936. An outbreak of acute swine erysipelas infection in turkeys. Jour. Am. Vet. Med. Assn. 88:475.

Breed, D. B.: 1943. Erysipelas in turkeys. A case report. Jour. Am. Vet. Med. Assn. 114:226. Am. Vet. Med. Assn. 126:135.

——, Hudson, C. B., Tudor, D. C., and Black, J. J : 1955. Erysipelas infection in poultry. Jour. Am. Vet. Med. Assn. 126:135.

Blaxland, J. D., Kershaw, G. F., and Howell, J.: 1949. Erysipelothrix rhusiopathiae infection in turkeys. Vet Record 61.350.

Bourgeois, E.: 1944. Stabchenrotlauf bei einer Wildente. Schweiz. Arch. Tierheilk. 86:32.

Boyer, C. I., Jr., and Brown, J. A.: 1957. Studies on erysipelas in turkeys. Avian Dis. 1:42.

Breed, F.: 1943. Erysipelothrix rhusiopathiae and Pasteurella avicida in chickens. Vet. Med. 38:430

Broll, H.: 1911. Über das Vorkommen von rotlaufähnlichen Bakterien beim Rinde und Huhne. Berliner tierarztl. Wochenschr. 27:41.

Brown, R. G., Doll, E. R., Bruner, D. W., and Kincaid, A. S.: 1949. A swine erysipelas outbreak in turkeys. Jour. Am. Vet. Med Assn 114:458.

Brunett, E. L., and Holstad, M. S.: 1945. Erysipelas in turkeys in New York State. Cornell Vet. 35:105

Byrne, J. L., Connell, R., Frank, J F., and Moynihan, I. W.: 1952. Studies of swine erysipelas. II. Cultural characteristics and virulence of strains of Erysipelothrix rhusiopathiae isolated in different regions in Canada. Canad. Jour. Comp. Med. 16:129.

Christiansen, M.: 1949. Sigdomme hos vildtlevende fugle. Dansk. Ornithol. Foren. Tidsskr. 43:189

Connell, R.: 1954 Erysipelothrix rhusiopathiae infection in a northern chipmunk, Eutamias minimus borealis. Canad Jour Comp. Med. and Vet. Sci. 18:22.

Cooper, M. S., Personeus, G. R., and Choman, B. R: 1954. Laboratory studies on the vaccination of mice and turkeys with an Erysipelothrix rhusiopathiae vaccine. Canad. Jour. Comp. Med and Vet Sci. 18.83.

——, Personeus, G. R., and Percival, R. C.: 1957. Laboratory studies on erysipelas. 4. Duration of immunity in turkeys vaccinated with an adsorbed bacterin. Poultry Sci. 36.266.

de la Villa, G. C.: 1954. Nota sobre la sensibilidad del pájaro al bacilo Erysipelothrix rhusiopathiae. Trab. Inst. Biol Anim. Madrid 2.350. (Abst. Vet. Bul. 5:789)

DeLay, P. D., and Koch, B.: 1950. Results of penicillin treatment during an outbreak of erysipelas in turkeys. Jour. Am. Vet. Med. Assn. 117.142.

de Mendonça Machado, A.: 1945. Infecção espontânea de columbideos pelo "B. rhusiopathiae suis." Repos Lab. Pat. Vet., Lisboa 6 63

Dickinson, E. M., Jensatd, A. G., Adler, H. E., Cooper, M., Babcock, W. E., Johns, E. E., and Bottorff, C. A.: 1955. The use of an Erysipelothrix rhusiopathiae bacterin for the control of erysipelas in turkeys. Proc. Am. Vet Med. Assn. 1953:370.

Doria, G.: 1943 Su un'enzoona di mal rossino nelle anitre. Nuova Vet. 21:72. (Abst. Vet. Bul. 16:59)

Dougherty, E., and Bruner, D. W.: 1954. Two cases of erysipelas in the White Pekin duck. Cornell Vet. 44:209.

Doyle, T. M.: 1960. Can swine erysipelas be eradicated? Epidemiological and immunological aspects. Vet. Rev. Annot 6 95.

Drake, C. H., and Hall, E. R.: 1947. The common rat as a source of Erysipelothrix rhusiopathiae. Am. Jour. Pub. Health 37.846.

Dunne, H. W., Belding, R. D., Newman, J. P., Johnston, R. L., and Stafseth, H. J.: 1951. Problem of erysipelas. Mich. St. Coll. Vet. 11:65.

Eber, A.: 1921. Geflugel-Rotlauf (Rotlauf-Septikämie der Vogel) Deutsch. tierärztl. Wochenschr. 29:295.

Elliott, H. B.: 1956. Erysipelas in caged turkeys. Jour. Amer. Vet. Med. Assn. 128:243.

Engel, J. A., and van der Maas, J. C. A.: 1955. Vlekziekte bij eenden. Tijdsch. Diergeneesk. 80:402.

Evans, W. M., and Narotsky, S.: 1954. Two field cases of *Erysipelothrix rhusiopathiae* infection in chickens. Cornell Vet. 44:32.

Frei, W., and Jezierski, A.: 1945. Chemotherapeutische Versuche mit Sulfanilamiden bei der Geflugelcholera- und Rotlaufinfektion der weissen Maus. Schweiz. Arch. Tierheilk. 87:136. (Abst. Vet. Bul. 17:204.)

Fuzi, M., and Pillis, I.: 1962. Die Differenzierung der Listeria monocytogenes und Erysipelothrix rhusiopathiae. Zentralbl. f. Bakt. I. Orig. 186:556

Galli, G.: 1953. Il mal rossino negli uccelli s osservazioni su un'enzoozia de bacillo del mal rossino in un allevamento de anatre. Clin. Vet., Milano 76.17.

Gifford, R., and Junghere, E.: 1946. A report on penicillin treatment of swine erysipelas in turkeys. Mich. St. Coll. Vet. 7:18.

Graham, R., Levine, N. D., and Hester, H. R.: 1939. *Erysipelothrix rhusiopathiae* associated with a fatal disease in ducks. Jour. Am. Vet. Med. Assn. 95:211.

Greener, A. W.: 1939. Infection of a peacock with *Erysipelothrix rhusiopathiae*, followed by a case of human erysipeloid. Brit. Jour. Dermat. 51:372.

Grenci, G. M.: 1943. The isolation of *Erysipelothrix rhusiopathiae* and experimental infection of turkeys. Cornell Vet. 33:56.

Grey, C. G.: 1947a. Effects of penicillin on *Erysipelothrix rhusiopathiae* and on mice infected with that organism. Vet. Med. 42:74.

——: 1947b. Penicillin in the treatment of *Erysipelothrix rhusiopathiae*-infected turkeys. Vet. Med. 42:177.

——: 1947c. Streptomycin in the treatment of *Erysipelothrix rhusiopathiae*-infected turkeys. Vet. Med. 42:216.

Gurova, E. I.: 1959. [Factors influencing the survival of pathogenic bacteria in soil.] Nauch. Trud. Ukrain. Inst. Eksp. Vet. 25:269. (Abst. Vet. Bul. 30.304.)

Hall, S. A.: 1963. A disease in pullets due to *Erysipelothrix rhusiopathiae*. Vet. Rec. 75:333

Hauser, A.: 1909. Bakteriologische Untersuchungen uber Geflugeldiphtherie. Zentralbl. f. Bakt. I. Orig. 48.555.

Heilman, F. R., and Herrell, W. E.: 1944. Penicillin in the treatment of experimental infections due to *Erysipelothrix rhusiopathiae*. Proc. Mayo Clin. 19:310.

Hoffman, H. A., and Hinshaw, W. R.: 1938. Erysipelas of turkeys. Poultry Sci. 17:443.

Horsemann, H.: 1938. Ein Beitrag zum Rotlauf bei Enten. Zeitschr. f. Infekt-Krankh. der Haustiere 53.106.

Hudson, C. B.: 1949. *Erysipelothrix rhusiopathiae* infection in fowl. Jour. Am. Vet. Med. Assn. 115:56.

——, Black, J. J., Buins, J. A., and Tudor, D. C.: 1952. Outbreaks of *Erysipelothrix rhusiopathiae* infection in fowl. Jour. Am. Vet. Med. Assn. 121:278.

Jármai, K.: 1920. Das Vorkommen der Rotlaufbazillen bei Vögeln. Allotorvosi Lapok. 1919. 8.57 (Abst. Berliner tierärzt Wochenschr. 36:17).

Jarosch, L. M: V 1905. Ueber Septikämie der Truthuhner. Oesterr. Monatschr. Tierheilk. 30.197.

Jerstad, A. C.: and Johns, E. E.: 1954a. Performance of a bacterin in the control of erysipelas in turkeys. Proc. Am. Vet. Med. Assn. 1954:333.

——, and Johns, E. E.: 1954b. Field trials of a bacterin for the control of erysipelas in turkeys. Jour. Am. Vet. Med. Assn. 125:288.

——, and Johns, E. E.: 1956. Control of an erysipelas outbreak in turkeys with a bacterin. Jour. Am. Vet. Med. Assn. 128:242.

——, and Johns, E. E.: 1957. Attempted control of erysipelas in turkeys with furazolidone. Jour. Am. Vet. Med. Assn. 130.99.

Junghere, E., and Gifford, R.: 1944. Three hitherto unreported turkey diseases in Connecticut erysipelas, hexamitiasis, mycotic encephalomalacia. Cornell Vet. 34:214.

Juslin, K. -E., and Stenberg, H.: 1954. Några jämförande försök mellan *Erysipelothrix rhusiopathiae* och *Listeria monocytogenes* isolerade från höns i Finland. Nordisk Veterinärmed. 6:457.

Karlson, A. G.: 1938. The cultural characteristics of *Erysipelothrix rhusiopathiae*. Jour. Bact. 35.205.

Konat, H.: 1945. Chemotherapy of swine erysipelas. Trials using sulfanilamide, sulfapyridine, and sulfathiazole in experimental infection of mice. Canad. Jour. Comp. Med. 9:135.

Kubuk, I.: 1912. Beitrag zur Epidemologie des Schweinerotlaufbazillus nach peroraler Infektion von Tauben. Wien. tierärzt. Monatsschr. 29.510.

Lindenmayer, J. E.: 1943. Swine erysipelas in turkeys in the state of Washington. Jour. Am. Vet. Med. Assn. 102.568

Lindenmayer, J. E., and Hamilton, C. M.: 1942. Treatment of swine erysipelas in turkeys with serum from a turkey infected with *Erysipelothrix rhusiopathiae*. Jour. Am. Vet. Med. Assn. 100:212.

Linsert, H.: 1944. Beiträge zu den Geflügelkrankheiten. II. Rotlaufinfektion bei Gansen. Tierärztl. Zentralbl. No. 2:25. (Abst. Vet. Bul. 17:527.)

Madsen, D. E.: 1937. An erysipelas outbreak in turkeys. Jour. Am. Vet. Med. Assn. 91:206.

Marinelli, G.: 1928. Le infezioni da mal rossino e da colera dei polli nel colombi tenuti a riso brillato. Folia Med. 14:1478.

Mitrovic, M., Matisheck, P. H., and Lynch, L. C.: 1961. Studies on the antigenicity in turkeys of an erysipelas bacterin made from Erysipelothrix rhusiopathiae strains 114 and 117. Avian Dis. 5:327.

Moore, E. N.: 1947. Diseases of turkeys in New York. Cornell Vet. 37:112.

————: 1951. Miscellaneous diseases of turkeys. Proc. Am. Vet. Med. Assn. 1951:206.

Moynihan, I. W., and Stovell, P. L.: 1954. The sensitivity of *Erysipelothrix rhusiopathiae* to antibiotics and its relation to chemotherapy. Proc. Am. Vet. Med. Assn. 1954:327.

Murase, N., Suzuki, K., and Nakahara, T.: 1959. Studies on the typing of *Erysipelothrix rhusiopathiae*. II. Serological behaviours of the strains isolated from fowls including those from cattle and humans. Jap. Jour. Vet. Sci. 21:177.

Narotsky, S., and Hawley, G. C.: 1949. Case reports. I. Swine erysipelas in turkeys infects man with erysipeloid. Mich. St. Coll. Vet. 9:50.

Nikolić, P., and Sepic, M.: 1961. Enzootija crvenog vetra kod podica. Vet. Glasn. 15.600. (Abst. Vet. Bul. 32:15.)

Orlandella, V.: 1955. Ricerche sull'azione dell'eritromicina nella infezione sperimentale del piccione da *E. rhusiopathiae*. Acta Med. Vet., Napoli 1:359.

Osebold, J. W., Dickinson, E. M., and Babcock, W. E.: 1950. Immunization of turkeys against *Erysipelothrix rhusiopathiae* with avirulent live culture. Cornell Vet. 40:387.

Peterson, E. H., and Hymas, T. A.: 1950. Diamond skin disease (chronic erysipelas) in a turkey. Jour. Am. Vet. Med. Assn. 117:465.

————, and Hymas, T. A.: 1952. Experimental and field immunization of turkeys against erysipelas. Poultry Sci. 31:94.

Pfaff, F.: 1921. Schweinerotlaufbakterien als Erreger einer chronischen Hühnerseuche. Zeitschr. f. Infekt-Krankh der Haustiere 22.293.

Poels, J.: 1919. Rotlauf bei Tauben und Enten und Stammunterschiede bei Rotlaufbazillen. Folia Microbiol. 5.1.

Prier, J. E., and Alberta, J. O.: 1960. The effects of aureomycin and of penicillin against *Erysipelothrix rhusiopathiae* in vitro and in vivo. Jour. Bact. 60:139.

Raines, T. V., and Winkel, F. H.: 1956 Erysipelas in pheasants. Jour. Am. Vet. Med. Assn. 129:399.

Reinhardt, R.: 1924. Septikämische Erkrankungen bei Schafen, verursacht durch Schweinerotlaufbazillen (including poultry). Monatshefte fur prakt. Tierheilk. 34:155.

Rosenwald, A. S.: 1940 Swine erysipelas in a week-old turkey poult. Jour. Am. Vet. Med. Assn. 96.268.

————, and Dickinson, E. M.: 1939. A report of swine erysipelas in turkeys. Cornell Vet. 29.61.

————, and Dickinson, E. M.: 1941. Swine erysipelas in turkeys. Am. Jour. Vet. Res. 2:202.

Rossi, G., and Pini, T.: 1953. Un interessante focolaio di mal rossino nei fagiani. Zooprofilassi 8:461.

Schipp, C.: 1910. Zur Biologie des Schweinerotlaufbazillus und zweier morphologisch gleicher Septikämeerreger. Deutsch. tierärztl. Wochenschr. 18:97.

Schlotthauer, C. F., and Thompson, L.: 1940 The occurrence of erysipelas in turkeys. Jour. Am. Vet. Med. Assn. 96:103.

Schmidt-Hoensdorf, F.: 1931. Rotlauferkrankungen bei Vögeln im Anschluss an Schweinerotlauf und Mäuseseptikämie. Deutsch. tierärztl. Wochenschr. 39:196.

Scholl, M. A., and Jacquart, M.: 1926. Enzootic chez la poule et le canard, provoquée par le bacille du rouget. L'Echo Vétérinaire 55:49.

Seibold, H. R., and Neal, J. E.: 1956. Erysipelothrix septicemia in the porpoise. Jour. Am. Vet. Med. Assn. 128.537.

Smith, H. W.: 1956. The chemotherapy of experimental *Erysipelothrix rhusiopathiae* infection in chicks and turkeys. Jour. Comp. Path. and Therap. 66:151.

Sparapani, G.: 1938. Casi interessanti di meningite bombare in polli Sussex millefiore causata dal bacillo del mal rossino dei suini. Coltura Avicola 1938:139, 158. (Abst. Int. Rev. Poultry Sci. 11:283)

Stiles, G. W.: 1944. Swine erysipelas organisms recovered from a brown rat (*Rattus norvegicus*). Am. Jour. Vet. Res. 5:245.

————: 1946. Observation of swine erysipelas in turkeys including the public health aspect and possible human cases). Jour. Am. Vet. Med. Assn. 109.65.

Szabó, B.: 1945. *Erysipelothrix rhusiopathiae* infection in pheasants (trans. title). Allatorv. Lapok. 17:100. (Abst. Vet. Bul. 16.387.)

Trbić, B., and Tadić, Z.: 1959 Erysipelothrix rhusiopathiae kao uzročnik oboljenja fazana. Vet. Glasn. 13.642. (Abst. Vet. Bul. 30:228.)

Urbain, A., Nouvel, J., and Roth, P.: 1943. Septicémie à bacille du rouget chez une perruche (*Palaeornis torquata*, L.). Bul. Acad. vét. France 16 (n.s.):156. (Abst. Vet. Bul. 15:72).

Valentin, F., Renault, L., and Joubert, L.: 1956-57. Contribution à l'étude du rouget aviaire Rouget enzootique chez le canard et le faisan. Bul. Soc. Sci. Vet. Lyon 58, 59:325.

van Bemmel, A. C. V., Peters, J. C., and Zwart, P.: 1960. Report on births and deaths occurring in the gardens of the Royal Rotterdam Zoo during the year 1958. Tijdschr. Diergeneesk. 85:1205.

van Dorssen, C. A., and Donkervoet, J.: 1953. Spontane *Erysipelothrix rhusiopathiae* infectie bij duiven. Tijdschr. v. Diergeneesk. 78:501.

Van Es, L., and McGrath, C. B.: 1936. Swine Erysipelas. Nebr. Agr. Exper. Sta., Res. Bul. 84.
——, Olney, J. F., and Biore, I. C.: 1945. The effects of penicillin on *E. rhusiopathiae*-infected pigeons. Nebr. Agr. Exper. Sta., Res. Bul. 141.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart.

Van Roekel, H., Bullis, K. L., and Clarke, M. K.: 1938. Erysipelas outbreaks in turkey flocks. Jour. Am. Vet. Med. Assn. 92:403.

Vianello, G.: 1938. Un'enzoozia di mal rosino nei fagiani. La Clinica Véterinaria 61:234.

Waller, E. F.: 1939. Erysipelothrix infection in a quail. Jour. Am. Vet. Med. Assn. 95 512.

Wellmann, G.: 1950. Rotlaufübertragung durch verschiedene Blutsaugende Insektenarten auf Tauben, Zentralbl. Bakt. I Abt. Orig. 155:109.

Werner, F.: 1932. Ein Beitrag Zum Geflügelrotlauf bei Enten. Deutsch. tierärztl. Wochenschr. 40:148.

White, E. G., and Henley, F. A.: 1942. *Erysipelothrix rhusiopathiae* associated with disease in ducks. Vet. Record 54:127.

Woodbine, M.: 1946. Chemotherapy of *Erysipelothrix rhusiopathiae* infections in mice. Vet. Jour. 102:88.
——, with assistance of Cheeseman, M. W.: 1947. Chemotherapy of *Erysipelothrix rhusiopathiae* infections in mice with streptomycin. Vet. Jour. 103:149.
——: 1950. *Erysipelothrix rhusiopathiae*. Bacteriology and chemotherapy. Bact. Rev. 14:161.

Zeiger, W.: 1952. Ein neuer Fall einer Rotlaufenzootie bei Enten. Deutsch. tierärztl. Wochenschr. 59:243.

Goose Influenza
(Septicaemia anserum exsudativa)

Goose influenza was first described in some detail by Riemer (1904) as the cause of a fatal disease of geese in Germany, and a specific bacterium was isolated from the affected birds. A similar microorganism was found in geese by Bugge (1908). This disease was later encountered in several German provinces by a number of investigators (Gerriets, 1953). Their data indicate that the disease was probably introduced into Germany by Russian and Polish geese. The disease of geese described by M'Fadyean (1902) in England was probably goose influenza. The hemorrhagic septicemia observed by Fiorentini (1896) in geese and swans was, however, due to a different organism, a bipolar staining, motile coliform rod, pathogenic for chickens, pigeons, rabbits, guinea pigs, geese, and ducks. The influenza bacterium, on the other hand, is pathogenic only for geese. Although this disease has not been reported in North America, the writer

(unpublished data) isolated an organism from a young goose in Illinois which was similar in preliminary tests to the causative agent of goose influenza.

Etiology. The organism causing goose influenza appears in the animal body as a small rod, frequently resembling a diplococcus. It varies in size from 0.5 to 1.5 by 0.5μ. It can always be found in large numbers in the heart blood, pericardial fluid, and fibrinous exudates. It is Gramnegative, forms no spores, and is nonmotile. While it can be rather easily isolated from an affected bird on plain nutrient agar, a medium containing hemoglobin is necessary for further transfers. Once adapted to artificial culture media, however, it will grow on plain agar.

Small, white, circular colonies are formed on gelatin plates, while in gelatin stabs a slight infundibuliform liquefaction occurs which becomes complete in several weeks. On nutrient agar, circular,

transparent, smooth, homogeneous, slightly viscid colonies are formed. They are grayish-white (bluish by transmitted light) at first, but become brownish-yellow with age. In broth a uniform turbidity is produced, and a slight sediment is formed after a few days. A pellicle may or may not be formed. Indol is usually not formed. Litmus milk is unchanged. Slight acid may be produced from dextrose but not from lactose. Hydrogen sulfide is formed. On coagulated blood serum, a yellowish-white growth appears, and the medium later becomes brownish and still later is liquefied. No growth occurs on Drigalski-Conradi or Endo media. The optimum temperature is about 37.5° C. No growth occurs below 15°, while an exposure to 56° C. for 5 minutes kills the organisms. Cultures are viable only a relatively short time.

The systematic position of this organism has varied with different authorities. Riemer (1904) named it *Bacillus septicaemiae anserum exudativae*, and it also been assigned to the genera *Shigella* (Bergey *et al.*, 1939, Smith, 1948) and *Hemophilus* (Frosch and Bierbaum, 1909; Löffler, 1910; Schlüter, 1936; Gerriets, 1953). However, Breed *et al.* (1957) called it *Pasteurella septicaemiae*, and this name is being used herein.

Symptoms. Both young and old geese are susceptible to the disease, while ducks, chickens, and turkeys are not affected. The disease generally appears in May and the first half of June, sometimes reappearing in the latter part of August and September. At the beginning of an outbreak, a few cases and some deaths are observed, but later a high percentage of the flock may become affected. At first the disease is found in the young geese, but as the epidemic progresses older birds are also affected. The mortality in young birds is especially high, often 70 to 90 per cent. The disease usually disappears from the flock in two to four weeks.

Few affected birds survive, and these generally exhibit paralysis or leg weakness for some time.

The first striking symptom is a decrease in appetite. The birds gradually become weaker, squat with ruffled feathers, and keep apart from the rest of the flock. According to Bugge, diarrhea sets in 12 to 24 hours before death. The birds strike with their legs and head, stagger while walking, and breathe rapidly. The beak is opened wide and a snoring noise is emitted. Death may come either gradually or quite suddenly. The course of the disease is ordinarily 2 to 5 days, although sometimes the birds may die within an hour after symptoms appear.

Pathology. In this disease two postmortem pictures have been described which are in partial agreement. Riemer described an exudative septicemia, while Bugge emphasized the inflammation of the air sacs and the lung lesions. Both Riemer (1904) and Eber (1921) found at necropsy only a slender, lightly adhering film on the liver surface, fine fibrinous threads on the epicardium, and a small amount of a turbid, serous exudate in the pericardium. No other morbid changes in the organs or tissues were demonstrated. According to Bugge, the roughened surface of the air sacs is covered by a yellowish film which can be easily pulled off, and the inner surfaces are covered by thick, coherent, whitish-yellow fibrin masses frequently distributed in reticular fashion. Since these masses may continue on to the lateral and posterior borders of the lung, numerous yellow nodules from the size of a pinhead to that of a pea are visible through the healthy rosy-red lung tissue at these places. The peripheral bronchi are filled with yellowish fibrinous masses. In a few cases hemorrhages in the intestinal mucosa and enlargement of the spleen, liver, and kidneys are observed.

Pathogenicity. The organism of goose influenza is pathogenic for geese, but rabbits, gray and white rats, mice, ducks, chickens, and pigeons are not affected by it. Guinea pigs may be killed by a large inoculum of the bacteria. Cultures lose

their virulence rather rapidly if they are not passed through geese regularly.

Diagnosis. In dead geese the disease is recognized rather easily by the characteristic fibrinous inflammation of the serous membranes or of the air sacs as well as by symptoms of a general septicemia. The diagnosis is confirmed by recognition of the bacteria in stained smears from affected areas or heart blood, or by isolation and identification of the causative microorganism.

Therapy. According to Gerriets (1953),

penicillin and sulfathiazole are effective in treating this disease.

Prevention. While vaccination with an autogenous bacterin may be attempted, the main emphasis in the control of goose influenza must be placed on the application of hygienic measures. Newly acquired birds should be held under quarantine. Sick birds should be separated from the rest of the flock or slaughtered, and all carcasses should be destroyed by burning. Houses and runs should be disinfected.

REFERENCES

Bergey, D. H., Breed, R. S., Murray, E. G. D., and Hitchens, A. P.: 1939. Manual of Determinative Bacteriology, 5th ed., Williams and Wilkins, Baltimore.

Breed, R. S., Lessel, E. F., Jr., and Clise, E. H.: 1957. Genus I. Pasteurella Trevisan, 1887. In Breed, R. S., Murray, E. G. D., and Smith, N. R., eds. Bergey's Manual of Determinative Bacteriology, 7th ed., Williams and Wilkins, Baltimore, pp. 395.

Bugge, G.: 1908. Ansteckende Luftsackentzündung der Gänse. Zeitschr. f. Infekt-Krankh. d. Haustiere 3:470.

Eber, A.: 1921. Gänse-Influenza (exsudative Septikämie und ansteckende Luftsackentzündung der Gänse). Deutsch. tierärztl. Wochenschr. 29:187.

Fiorentini, A.: 1896. Hämorrhagische Septikämie der Schwane. Zentralbl. f. Bakt. etc. I. Orig. 19:932.

Frosch, P., and Bierbaum, R.: 1909. Ueber eine durch den Bacillus septicaemia anserum exsudativae (Riemer) bedingte Gänseseuche. Zentralbl. f. Bakt. I. Orig. 52:455.

Gerriets, E.: 1953. Antibiotica- und Chemotherapie bei einem Fall von Septicaemia anserum exsudativa. Berliner Münchener tierärztl. Wochenschr. 66:261.

Löffler, F.: 1910. Ueber eine in Jahre 1904 in Klein-Kiesow bei Greifswald beobachtete Gänseseuche. Arzb. f. wiss. u. prakt. Tierheilk. 38:289. Suppl. Bd.

M'Fadyean, J.: 1902. A remarkable outbreak of goose septicaemia. Jour. Comp. Path. and Therap. 15:162.

Miessner, H., and Berge, R.: 1923. Septicaemia anserum exsudativa (Gänseinfluenza). Deutsch. tierärztl. Wochenschr. 31:539.

Fröscholdt: 1919. Gänseinfluenza. Berliner tierärztl. Wochenschr. 33:261.

Reinhardt, R.: 1921. Untersuchungen über die Septicaemia anserum exsudativa. Zeitschr. f. Infekt-Krankh. d. Haustiere 21:257.

Riemer: 1904. Kurze Mitteilung über eine bei Gänsen beobachtete exsudative Septikämie und deren Erreger. Zentralbl. f. Bakt. I. Orig. 37:641.

Schlüter, W.: 1936. Beitrag zur serologischen Differenzierung hämoglobinophiler Bakterien. Zentralbl. f. Bakt. etc. I. Orig. 136.362.

Smith, F.: 1948. Genus II. Shigella Castellani and Chalmers. In Breed, R. S., Murray, E. G. D., and Hitchens, A. P.: Bergey's Manual of Determinative Bacteriology, 6th ed., Williams and Wilkins, Baltimore, p. 535.

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16

Streptococcosis, Staphylococcosis, Arthritis, Coli-granuloma (Hjärre's Disease), and Colibacillosis

Avian Streptococcosis

Streptococcic infections occur sporadically in fowls. The disease has been reported in many countries of the world. It has been described as an acute septicemia of fowls (Nórgaard and Mohler, 1902; Greve, 1908; Magnusson, 1910; Hudson, 1933; Edwards, 1934; Buxton, 1952). Others have described chronic infections caused by streptococci (Dammann and Manegold, 1905; Kernkamp, 1927; Edwards and Hull, 1937). Streptococci have been isolated from an acute disease in young geese and ducklings by Hodosy (1944) from turkeys by Volkmar (1932), and from pigeons by Madej (1961).

Etiology. The most common causative agent is *Streptococcus gallinarum*. Merchant and Packer (1961) have described this organism as Gram-positive, occurring in chains of 6 to 8 cells. Long chains are found in fluid media, while diplococcoid forms occur on solid media. Some strains

have capsules. On agar, young colonies are white and gelatinous. Older colonies have bluish borders and brown centers. Beta hemolysis is observed on blood agar, and leucocidin is also produced. *Streptococcus zooepidemicus* also may be isolated occasionally from chickens (Edwards and Hull, 1937; Packer, 1951; Buxton, 1952; Agrimi, 1956; Sato *et al.*, 1960). This species can be distinguished from *Streptococcus gallinarum* by its ability to ferment sorbitol. *Streptococcus gallinarum* does not ferment this sugar. Agrimi (1956) isolated *Streptococcus faecalis* from 5 of 6 cases of acute septicemia in fowls. *S. faecalis* has been isolated from cases of bacterial endocarditis by Gross (1962).

Transmission. The disease has been reproduced with strains isolated from acute outbreaks. The route of infection in the natural disease is not known, although Hudson (1933) suggested that the organism might be introduced by way of the respiratory tract. He also mentioned that

carriers may exist after an outbreak and that these might serve to introduce the *disease into a susceptible flock.*

Pathogenicity. Chickens are the natural host, but other species, namely rabbits, pigeons, ducks, geese, turkeys, and white mice, are susceptible. Guinea pigs are resistant.

Symptoms and lesions. In acute cases death may occur without definite symptoms. Lesions consist of congestion and enlargement of the parenchymatous organs. In the chronic form, depression and debility are prominent symptoms. On necropsy there may be necrotic foci in the liver, a fibrinous exudate in the abdominal cavity, and an enteritis.

Mortality. In the flock described by

Nörgaard and Mohler (1902) the loss was *over* 90 per cent of the birds. Losses in other outbreaks mentioned were less severe, and in chronic cases the mortality was considerably less.

Diagnosis. Isolation of *Streptococcus gallinarum* or *Streptococcus zooepidemicus* constitutes a diagnosis. The organism may be isolated from the blood and parenchymatous organs, or in chronic cases from localized areas of infection.

Treatment. While there have been *no* reports of attempted treatment with the sulfonamides and antibiotics, some of these might be tried in outbreaks of streptococcosis, particularly where valuable breeding stock is involved.

REFERENCES

Agrimi, P.: 1956. *Studio sperimentale su alcuni focolai di streptococcosi nel pollo.* Zooprofilassi 11:491.

Buxton, J. C.: 1952. *Disease in poultry associated with Streptococcus zooepidemicus.* Vet. Record 64:221.

Dammann, C., and Manegold, O.: 1905. *Die Schlafkrankheit der Hühner.* Deutsch. tierärztl. Wochenschr. 13:577.

Edwards, P. R.: 1934. *Characters of hemolytic streptococci isolated from pathological conditions in fowls.* Jour. Comp. Path. and Therap. 47:152.

———, and Hull, F. E.: 1937. *Hemolytic streptococci in chronic peritonitis and salpingitis of hens.* Jour. Am. Vet. Med. Assn. 91:656.

Greve, L.: 1908. *Beitrag zur Kenntnis der Streptokokken-Krankheit (Schlafkrankheit) der Hühner.* Deutsch. tierärztl. Wochenschr. 15:213.

Gross, W. B., and Domermuth, C. H.: 1962. *Bacterial endocarditis of poultry.* Am. Jour. Vet. Res. 23:320.

Rodosy, J.: 1944. *(Streptococcosis in young geese and ducks.)* Allatorv. Lapok., p. 37. (Abst Vet. Bul. 16:426.)

Hudson, C. B.: 1933. *A specific infectious disease of chickens due to a hemolytic streptococcus.* Jour. Am. Vet. Med. Assn. 82:218.

Kernkamp, H. C. H.: 1927. *Idiopathic streptococcic peritonitis in poultry.* Jour. Am. Vet. Med Assn. 70:585.

Madej, E.: 1901. *Obserwacje nad streptokokkoza golebi.* Med. Wet. 17:713.

Magnusson, H.: 1910. *Ueber eine für Europa neure Huhnerseuche. Apoplektische Septikamie der Hühner.* Zentralbl. f. Bakt. I, Orig. 56:411.

Merchant, I. A., and Packer, R. A.: 1961. *Streptococcus gallinarum.* In: Veterinary Bacteriology and Virology. Sixth edition. Iowa State University Press, Ames, Iowa. P. 282.

Nörgaard, V. A., and Mohler, J. R.: 1902. *Apoplectiform septicemia in chickens.* Bur. Anim. Ind., U.S.D.A., Bul. 36.

Packer, R. A.: 1951. Personal communication.

Sato, G., Miura, S., and Ushijima, J.: 1960. *An outbreak of hemolytic-streptococcal infection among chickens of a flock. II. Characters of the isolated streptococcus.* Jap. Jour. Vet. Res 8:285.

Volkmar, F.: 1932. *Apoplectiform septicemia in turkeys.* Poultry Sci. 11:297.

Avian Staphylococcosis

Staphylococci are occasionally isolated from chronic or acute conditions of fowl. The organism has been isolated from cases of arthritis (Hole and Purchase,

1931; Jungherr, 1933; Gwatkin, 1940; Jungherr and Plastridge, 1941; Rowlands and Smith, 1945; Fahey, 1954). Staphylococci have been isolated from cases of

vesicular dermatitis (Hoffman, 1939), omphalitis (Williams and Daines, 1942), keel bursitis (Van Ness, 1946), and synovitis (Hinshaw and McNeil, 1952; and Madsen, 1942). Other reports of staphylococcic infections in fowl have been made (Lucet, 1892; Freese, 1907; Eber, 1921; Hasenkamp and Sachwch, 1914; Schlegel, 1922; Reinhardt, 1922; van Heelsbergen, 1919, 1929; Seetharaman and Sharma, 1949; Dal Santo, 1959; Sato et al., 1961). Outbreaks of "wing-tip" gangrene due to Staphylococcus aureus and Clostridium welchii Type D have been described in Italy (Rossi, 1956; Mondini and Quaglio, 1956, 1959; Zoletto, 1957). Rossi describes the acute form of the disease as a catarrhal conjunctivitis, crust formation on the skin of the back, joint lesions, and purulent panophthalmia and death in 8–10 days. Mortality was 50 per cent in 5 flocks of young chicks. Mondini and Quaglio (1959) state that osteoarthritis is a prominent feature in the majority of cases.

Etiology. The causative organism is Staphylococcus aureus. The pathogenic strains coagulate rabbit plasma, produce beta hemolysis on blood agar, ferment lactose and manitol, liquefy gelatin, and produce necrosis in the skin of rabbits.

Susceptible species. Staphylococcus aureus has been isolated from geese (Lucet, 1892; Rowlands and Smith, 1945), from pheasants (Hole and Purchase, 1931), from turkeys (Jungherr, 1933), and from chickens. Rabbits are the most susceptible of the laboratory animals (Merchant and Packer, 1961).

Transmission. Pathogenic strains of Staphylococcus aureus produce a fatal septicemia in young chicks when given intravenously. Occasionally an arthritic lesion is produced (Jungherr and Plastridge, 1941). In the natural disease it is believed that injuries serve as portals of

entry for Staphylococcus aureus. Hinshaw and McNeil (1952) could reproduce the disease in turkeys only by intravenous inoculation.

Symptoms and lesions. In the acute disease, which is fatal within several days, there are symptoms of diarrhea, depression, and joint swelling. The parenchymatous organs are found congested and swollen at necropsy. In the chronic cases the outstanding symptoms are a hobbling gait, frequent sitting or reluctance to move, enlarged joints, gradual emaciation, and finally death. The pathology is primarily an arthritis and synovitis. The tibiometatarsal joint is most frequently involved, although others may be affected. Initially there is a swelling of the affected joint, which is due to a serous inflammatory exudate. Later this exudate becomes caseous in character, and the joint swelling becomes firm.

Diagnosis. Isolation of Staphylococcus aureus from the blood stream or from affected joints of sick birds constitutes a diagnosis. The pathogenicity of the organism isolated should be determined by the coagulase test and the presence of alpha hemolysin.

Treatment and prevention. Autogenous bacterins, sulfadiazine, and sulfanilamide have not been effective (Jungherr and Plastridge, 1941; Rowlands and Smith, 1945). Broad-spectrum antibiotics were found effective in treating turkey poults suffering from arthritis (Fahey, 1954).

Since injuries may serve as atria of infection, sharp objects, such as nails, glass, and barbed wire, should be removed from the premises. Wire floors should be examined for sharp ends. When anti-pick devices must be placed on the birds, some precaution should be taken to avoid introducing infection.

REFERENCES

Dal Santo, F.: 1959. La stafilococcosi come malattia neonatale del pulcino. Zooprofilassi 14:653.
Eber, A.: 1921. Seuchenhafte Staphylokokkenkrankheit (ansteckende Knochen- und Gelenkentzündung) des Geflügels. Deutsch. tierärztl. Wochenschr. 29:119. (Cited by Jungherr, 1933)
Fahey, J. E.: 1954. An outbreak of staphylococcal arthritis in turkey poults. Poultry Sci. 33:661.

FIG. 16.1 — Periarticular abscesses caused by B. arthropyogenes. Spontaneous case. (Courtesy P. Nobrega, Arq. Inst. Biol., São Paulo.)

condition was reproduced in poults by intravenous or foot pad inoculation with culture material. Paratyphoid organisms may

produce joint infection in pigeons (Fenstermacher, 1952).

REFERENCES

Beaudette, F. R.: 1936. Arthritis in a chick caused by *Salmonella pullorum*. Jour. Am. Vet. Med. Assn. 89:89.

Boyer, C. I., Jr., Bruner, D. W., and Brown, J. A.: 1958. A streptobacillus, the cause of tendon-sheath infection in turkeys. Avian Dis. 2:418.

Carnaghan, R. B. A. and Sojka, W. J.: 1958. Arthritis in chicks due to a variant strain of *S. pullorum*. Vet. Rec. 70:615.

Fenstermacher, R.: 1952. Paratyphoid infections. In: Diseases of Poultry. Third edition. H. E. Biester and L. H. Schwarte. Iowa State University Press, Ames, Iowa. P. 277.

Ferguson, A. E., Connell, M. C., and Truscott, R. B.: 1961. Isolation of *Salmonella pullorum* from the joints of broiler chickens. Canad. Vet. Jour. 2:145.

Gallo, P.: 1942. Estudios sobre una nueva entidad nosográfica: "La artritis infecciosa de las aves, y su agente": "La Escherichia Venezuelensis," n. sp. Rev. Med. vet. Parasit., Caracas. 4:3. (Abst Vet. Bul. 17:557.)

Nobrega, P.: 1940. Artrite em galinha, produzida por "*Bacterium arthropyogenes*." Arq. Inst. Biol., São Paulo. 11:323.

Reis, J.: 1942. Artrite em galinha produzida por *Salmonella pullorum*. Arq. Inst. Biol., São Paulo 13:115.

Coli-granuloma (Hjärre's Disease)

A condition in chickens characterized by granulomatous lesions in the wall of the digestive tract and in the liver has been described by Hjärre and Wramby (1945). It is a fairly common disease in Sweden and is important because it can be confused with *tuberculosis*. Granulomas similar in morphology and distribution, but of unknown etiology, have been observed in the United States (Bennett *et al.*, 1951; Jungherr, 1949; Snoeyenbos, 1952). In Canada, Schofield (1947) ob-

served a disease in turkeys which he considered to be coli-granuloma. Wickware (1948), also from Canada, found the disease in chickens. Savage and Isa (1956) observed the disease in 11 cases, 10 of them being in turkeys. Hamilton and Conrad (1958) diagnosed the disease in 2 outbreaks involving 1,000 and 2,000 chickens. Mortality was 75 per cent and a mucoid *E. coli* was isolated; however, they were unable to reproduce the condition.

Etiology. Hjärre and Wramby (1945)

found a coliform bacterium as the causative agent. It is a Gram-negative, capsulated organism which forms mucoid colonies. The mucoid, M-form, of the organism may dissociate in culture into an S-form and a noncapsulated R-form. The agglutination type of the M-form corresponds with Kauffman's M-agglutination in coliform bacteria. Ulbrich (1951) studied 22 mucoid strains of *E. coli* isolated from cases of coli-granuloma in Germany and Sweden. Sixteen belonged to O group 4, four to O group 8, and two to O group 16. Six capsular (K) antigens were found among the cultures: K1, K6, K7, K8, K9, and K12. H antigen was not demonstrated among these cultures.

Pathogenicity. The disease is apparently only slightly infectious. Feeding infected material has not produced the disease. However, intramuscular injections of infected tissue suspensions or intravenous injections of pure cultures of the coliform organism have resulted in typical morphological lesions in many of the experimental chickens (Hjärre and Wramby, 1945). The bacterium is also pathogenic for white mice and rabbits.

Symptoms and lesions. Affected birds become thin and depressed. The organs most commonly involved are the ceca and liver, although granulomae may occur at

FIG. 16.2 — Longitudinal section of cecum through a granuloma. Enlarged. (Hjärre, State Vet. Med. Inst., Stockholm, Sweden.)

FIG. 16.3 — Ceca containing granulomae (Hjärre, State Vet. Med. Inst., Stockholm, Sweden.)
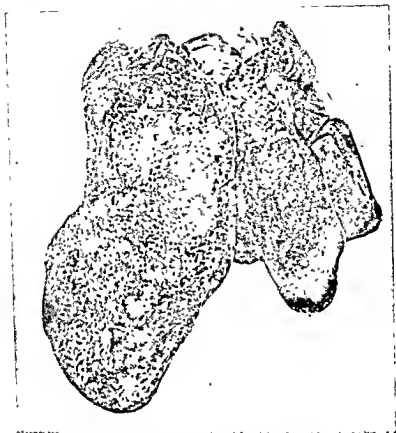
FIG. 16.4 — Liver from chicken with coli-granuloma. (Hjarre, State Vet. Med. Inst., Stockholm, Sweden.)

any place along the digestive tract and in the spleen and bone marrow (Hjarre and Wramby, 1945). It is common to find only isolated granulomae on the wall of the ceca (Fig. 16.2), although the lesions may involve the entire wall (Fig. 16.3). When the disease involves the liver, there is enlargement and large, irregularly shaped areas of necrosis (Fig. 16.4).

Diagnosis. Coli-granuloma may be confused with tuberculosis, although distribution of lesions, microscopic pathology, and the absence of acid-fast bacilli serve in differentiation. Old coli-granulomae cannot be distinguished from tuberculosis on the basis of morphology. In the early stages the coli-granulomae differ from tubercles in quantity, size, and appearance of giant cells (Hjärre and Wramby, 1945). Isolation of a mucoid, capsulated coliform bacterium which will reproduce the disease in experimental chickens would constitute a diagnosis.

REFERENCES

Bennett, P. C., Switzer, W. P., and Jones, L. D.: 1951. Report of the Iowa Veterinary Diagnostic Laboratory (1950–51). Iowa State Coll., Bul. 49.62.

Hamilton, C. M., and Conrad, R. D.: 1958. Extreme mortality in Hjarre's disease (coli-granuloma) in chickens. Jour. Am. Vet. Med. Assn. 132.84–85.

Hjärre, A., and Wramby, G.: 1945. Undersökningar över en med specifika granulom förlöpande hönssjukdom orsakad av mukoida kolibacterier (Koli-granulom). Skand. Veterinärtidskr. 35·149.

Jungherr, E. L.: 1952. Personal communication.

Savage, A., and Isa, J. M.: 1956. A note on Hjärre's disease in Manitoba. Cornell Vet. 46.579.

Schofield, F. W.: 1947. Hjärre and Wramby disease in turkeys (coli-granuloma). Canad. Jour. Comp. Med. 11:141.

Snoeyenbos, G. H.: 1952. Personal communication.

Ulbrich, Von F.: 1951. Untersuchungen über die Antigenstruktur von B. coli, die aus Koli-granulomen von Hühnern isoliert wurden. Monatshefte für Veterinärmed. 6:595.

Wickware, A. B.: 1948. Infectious granuloma of fowls. A preliminary note. Canad. Jour. Comp. Med. 12:294.

Avian Colibacillosis

Escherichia coli frequently can be isolated from diseased fowl. It is usually considered to be a secondary invader, although it has been incriminated as the cause of death in some flocks.

A number of investigators have reported on the isolation of coliform bacteria from septicemic infections of fowl (Lignières, 1894; Martel, 1897; Claussen, 1907; Zeiss, 1914; Palmer and Baker, 1923; Davis, 1938; Twisselmann, 1939; Gurumurthi and Panduranga Rao, 1962). Some have suggested that *E. coli* can cause a septicemia if the resistance of the bird is weakened by hunger, thirst, extremes of temperature, and low vitality (Claussen, 1907; Davis, 1938). Coliform organisms have been isolated from eggs laid by hens infected with *Salmonella pullorum* (Garrard, 1946). Bueno (1940) found the coliform organisms common in the lower digestive tract of chickens. Isolations of *E. coli* are made frequently from cases of pericarditis associated with so-called air sac infection in broilers (Gross, 1956; Biddle and Cover, 1957). Gross and Siegel (1959) produced peritonitis in chickens by intraperitoneal inoculation of a pathogenic strain of *E. coli* together with sterile yolk. Edwards and Ewing (1954) studied 50 cultures of *E. coli* isolated from internal organs of fowl and found them to belong to serotype 02:K1:H5. This type was not found among 200 cultures of *E. coli* from the intestines of fowl or other animals. Gross (1956) found this serotype to produce pericarditis and air sac infection. Sojka and Carnaghan (1961) studied 797 *E. coli* strains from poultry. Over 60 per cent belonged to the serological "O" groups, 02, 078, and 01.

Reproduction of *E. coli* infection has been attempted by some investigators.

Davis (1938), using both healthy chicks and chicks of low vitality, was able to demonstrate pathogenicity of the organism for the weak chicks, but low pathogenicity for the healthy chicks. Osborne *et al.* (1946) made inoculations of several strains of *E. coli* intraperitoneally into twenty-four young chicks. The chicks sickened within 24 hours, although all but two recovered. Gross (1956) found that five serological types of *E. coli* were capable of producing pericarditis following air sac inoculation or aerosol exposure. A combination of other agents, such as *Mycoplasma gallisepticum*, increased the pathogenicity of *E. coli*.

Symptoms and lesions. In the disease described by Davis (1938) the young chicks were weak and had a pasty vent. At necropsy about 50 per cent of the chicks had enlarged livers with necrotic foci. A few of the livers were covered with a gelatinous exudate. All had unabsorbed yolks. The disease described by Claussen (1907) affecting adult chickens was similar to fowl cholera. The birds were depressed, refused to eat, and some had diarrhea. Necropsy of affected chickens revealed petechial hemorrhages and hepatic congestion.

Diagnosis. Isolation of *Escherichia coli* from the blood and parenchymatous organs of killed, sick fowl, and the elimination of other possible causes are necessary for an accurate diagnosis. Some effort should be made to determine the pathogenicity of the isolated organism for the species from which it was cultured, and if possible to have the organism typed. Sojka and Carnaghan (1961) found all strains sensitive to furazolidone. An increasing number of strains were found resistant to tetracycline.

REFERENCES

Biddle, E. S., and Cover, M. S.: 1957. The bacterial flora of the respiratory tract of chickens affected with chronic respiratory disease. Am Jour. Vet. Res. 18:405.

Bueno, R. C.: 1940. Identificacao do grupo coli-aerogenes em aves. Distribuicao e frequencia. Arq. Inst. Biol., São Paulo. 11 69.

Claussen, L.: 1907. Über Kolibakteriensepukämie bei Huhnern als Transportkrankheit. Zeitschr. f. Infektionskr. d. Haustiere 3.69.

Davis, C. R.: 1938. Colibacillious in young chicks. Jour. Am. Vet. Med. Assn. 92:518.

Edwards, P. R., and Ewing, W, H: 1954. Studies on a coliform type isolated from the organs of fowls Cornell Vet. 44:50.

Garrard, E. H.: 1946. Coliform contamination of eggs. Canad. Jour. Res. 24:121.

Gross, W B: 1956 *Escherichia coli* as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Poultry Sci. 35 765.

——, and Siegel, P. B.: 1959. Coliform peritonitis of chickens. Avian Dis. 3:370.

Gurumurthi, V, and Panduranga Rao, P.: 1962. Coli-bacillosis in brooder chicks, Indian Vet. Jour. 39.66.

Lignières, M. J : 1894. Septicémie à coli-bacille chez la poule. Compt. rend. Soc. de biol. 46:135.

Martel, M.: 1897. Maladie à coli-bacille de la poule et de la dinde. Compt. rend. Soc. de biol. 49:500.

Osborne, J. C., Witter, J. F., and Hitchner, E. J.: 1946. A comparative study of cultures of microorganisms involved in chronic colibacillosis in fowl. Mich. St. Coll. Vet. 6:25.

Palmer, C. C., and Baker, H. R : 1923. Studies on infectious enteritis of poultry caused by *Bacterium coli communis*. Jour. Am. Vet. Med. Assn. 63 85.

Sojka, W. J., and Carnaghan, R. B. A.: 1961. *Escherichia coli* infections in poultry. Res. Vet. Sci. 2 340

Twisselmann, N. M.: 1939 An acute infectious disease of pullets apparently caused by *Escherichia coli communis*. Jour. Am. Vet. Med. Assn. 94:235.

Zeiss, H.: 1914. Kolisepukämie bei Huhnern. Arch. f. Hyg. 82.27.

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17

Ulcerated Enteritis, Quail Disease

This enteric disease is primarily of importance to those engaged in rearing quail, but increasing reports of morbidity and mortality from this disease in other avian species points up the need for diagnosis and treatment.

INCIDENCE

Durant and Doll (1941) stated that the disease was reported in the common Bobwhite, the California quail, the mountain quail, the sharptail grouse, the Gambel's quail, the European partridge, the chukar partridge, the wild turkey, and the domestic fowl. Levine (1932) reported on ulcerative enteritis of epidemic proportions in ruffed grouse and transmitted the disease to quail by feeding them flies that had been permitted to feed on intestinal contents from the affected grouse. Fenstermacher and Doyle, in the report by Schillinger and Morley (1934), found ulcerative enteritis in young turkeys and indicated it could be a serious problem. Bullis and Van

Roekel (1944) reported finding ulcerative enteritis in domestic turkeys and they submitted intestines to J. E. Shillinger of the U.S.D.A. who succeeded in infecting quail with this material. Glover (1951) and Peckham (1963) observed ulcerative enteritis in pigeons. Shillinger and Morley (1934) reported observing ulcerative enteritis in young wild turkeys. They also described the disease in domestic chicks and pullets, and they infected quail with intestinal contents from these chickens. These workers were able to transmit ulcerative enteritis from quail to wild turkey poults. Peckham (1959, 1960) reported transmission of the disease to quail by oral inoculation of liver suspensions or intestinal contents from affected chickens or turkey poults. Buss *et al.* (1958) found the disease in pheasants and in wild quail taken in southern Washington. Most of the reports concerning ulcerative enteritis have originated in the United States but Harris (1961) was the first to record an outbreak of ulcerative
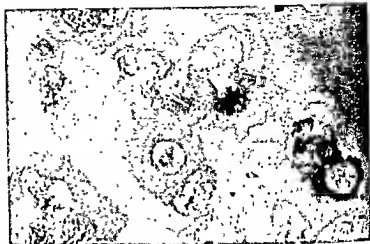
enteritis occurring in quail in Great Britain.

SIGNS

In quail the disease may assume an acute or chronic form. In young quail the losses may approach 100 per cent in a matter of a few days. Outbreaks may occur in birds reared on wire but are more common in birds reared on litter or ground. There may be no premonitory signs in birds dying suddenly. Such birds are usually well muscled and fat with feed still in the crops. A very striking characteristic of quail affected with ulcerative enteritis is a watery dropping containing urates (Fig. 17.1). In transmission studies the first sign of infection is a change from the normal dry and firm dropping to that of watery consistency. As the disease progresses, infected quail become listless, humped up, with the eyes partly closed and the feathers dull and ruffled. In birds affected a week or more there is a marked wasting of the pectoral muscles and eventually extreme emaciation occurs. There is a marked drop in feed consumption. The course of the disease following experimental infection is approximately three weeks with the peak of mortality occurring between 5 and 14 days following inoculation. Bass (1941b) stated that when wide distribution had occurred in quail stock, it took from 6 to 10 months for all infected birds to die out.

GROSS LESIONS

Gross lesions in quail are variable depending upon the time elapsing between infection and death. Birds dying suddenly may have a marked hemorrhagic enteritis in the upper portion of the intestine. Small punctate hemorrhages may be visible in the intestinal wall. The liver and spleen may show no gross changes in acute cases. In chronic cases the lesions become more prominent and extensive. Ulcers may occur in any portion of the intestine and ceca (Fig. 17.2). Small yellow foci with a hemorrhagic border may be seen both from the serosal and mucosal surfaces (Figs. 17.3 and 17.4). As the ulcers increase in size the hemorrhagic border tends to disappear. The ulcers may be lenticular or roughly circular in outline sometimes coalescing to form large necrotic diphtheritic areas. The lenticular shape is more common in the upper portion of the intestine. The ulcers may be deep in the mucosa or in older lesions they may be superficial and have raised edges (Fig. 17.5). Ulcers in the ceca may have a central depression filled with dark staining material that cannot be rinsed away readily. Perforation of the ulcers commonly occurs resulting in peritonitis and intestinal adhesions.

Liver lesions vary from a light yellow mottling to large irregular yellow areas of necrosis along the edges of the liver. Other
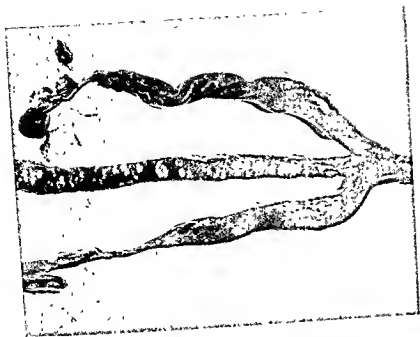
FIG. 17.2 — Quail intestine with oval ulcerations visible through the serosa. (Courtesy P. P. Levine, Dept. Avian Diseases, Cornell University.)

FIG. 17.3 — Chicken intestine. Note lenticular ulcers with a hemorrhagic border visible through the serosal surface.

FIG. 17.4 — Chicken intestine. Same as Fig. 17.3. Note ulcerations on mucosal surface.

FIG. 17.5 – Poult intestine with craterlike ulcerations from a natural case of ulcerative enteritis.

FIG. 17.6 – Combined ulcerative enteritis and E. brunetti in the intestine of a chicken. Note small ulcers in ceca and rectum. Diphtheritic membrane is due to coccidial infection.

liver lesions are disseminated grey foci or small yellow circumscribed foci which are sometimes surrounded by a light yellow halo effect. The spleen may be congested, enlarged, and hemorrhagic. Gross lesions are absent in the other organs. Peckham (1959) described liver, cecal, and intestinal lesions in chickens dying from ulcerative enteritis and noted that the disease in chickens is usually preceded or accompanied by coccidiosis (Fig. 17.6). Bullis and Van Roekel (1944) noticed that coccidiosis and ulcerative enteritis were often associated in poults. Witter (1952) reported complications in broiler flocks affected with coccidiosis. He found intestinal and cecal ulcerations similar to those of ulcerative enteritis in quail and, in addition, swollen

livers with pale, marginal necrotic areas. Gray et al. (1954) noted intestinal and cecal ulcerations with large areas of necrosis in the livers of chickens with hemorrhagic disease. All of these flocks had been treated with coccidiostats. Jungherr (1955) indicated that ulcerative enteritis was increasing in turkey poults and chickens (Fig. 17.7). He stated that some cases of coccidiosis did not respond to the usual coccidiostats, because of complications with ulcerative enteritis, and only when the treatment was changed to antibiotics or furazolidone did the flock improve. Chickens with ulcerative enteritis manifest signs similar to those of coccidiosis. As a result of this, flock owners usually medicate with a coccidiostat. Rosen and Bischoff (1949) re-
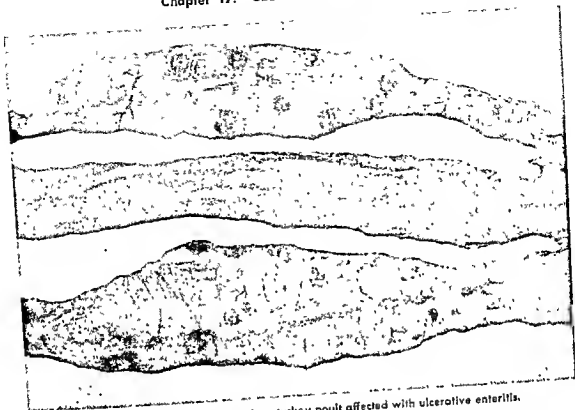
FIG. 17.7 — Cecal ulcerations in a turkey poult affected with ulcerative enteritis.

ported that 1 per cent sulfamethazine and 0.25 per cent sulfaquinoxaline in mash fed for 3 days had no effect on the course of ulcerative enteritis in quail. Churchill and Coburn (1915) reported on the failure of sulfonamide therapy against ulcerative enteritis. In chickens the liver lesions were either small, discrete, yellow, necrotic foci or large, irregularly shaped light yellow areas on the border of the liver (Fig. 17.8). Ulcerations were present in either the ceca, the intestine, or both. The combination of cecal and liver lesions makes the differentiation of this form of ulcerative enteritis and blackhead necessary. This can be done by histological examination or by demonstrating the ulcerative enteritis bacillus in liver smears.

Peckham (1960) described an unusual lesion of ulcerative enteritis in poults characterized by a necrotic, diphtheritic membrane occupying the middle third of the intestine. This combination of necrosis and sloughing of the intestinal mucosa appeared similar to the lesion produced by *Eimeria brunetti* infection in chickens (Fig. 17.9).

HISTOPATHOLOGY

A detailed description of the histopathology of ulcerative enteritis in quail was given by Durant and Doll (1941). Intestinal sections from acute cases revealed desquamation of mucosal epithelium, edema of the intestinal wall, vascular engorgement, and lymphocytic infiltration. The lumen of the intestine contained desquamated epithelium, blood cells, and fragments of mucosa. Early ulcers were small hemorrhagic necrotizing areas involving the villi and penetrating into the submucosa. Cells adjacent to these areas exhibited coagulation necrosis with karyolysis and karyorrhexis. Lymphocytic and granulocytic infiltration occurred in the area adjacent to the necrosis. Small clumps of bacteria were present in the necrotic tissue. Older ulcers appeared as thick masses of

FIG. 17.8 — Liver from a chicken with ulcerative enteritis. Note necrotic foci, some of which have a light yellow halo effect.
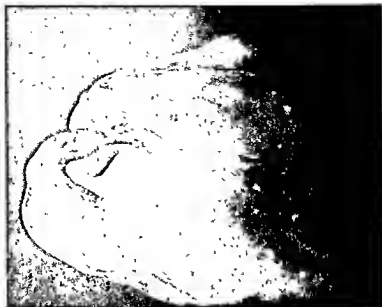
FIG. 17.9 — A necrotic diphtheritic membrane in the intestine of a poult caused by "quail disease."

granular acidophilic coagulated material mixed with cellular detritus and bacteria (Fig. 17.10). Granulocytes and lymphocytes infiltrated the area surrounding the ulcer (Fig. 17.11). In the submucosa and muscularis small blood vessels near the ulcers were occasionally occluded by bacteria.

ETIOLOGY

The isolation and identification of the etiological agent has been the subject of several investigations and, to date, confirmation of these reports is lacking. Morley and Wetmore (1936) reported the isolation

FIG. 17.10 — Transverse section of quail intestine with a large ulceration. ×80.
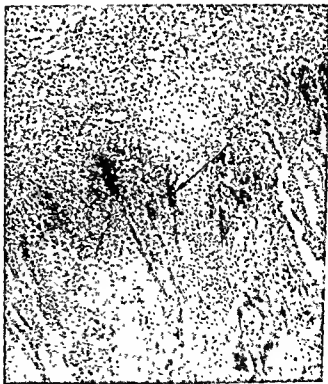
FIG. 17.11 — Higher magnification of area indicated by arrow in Fig. 17.10. Note cellular infiltration and dark linear masses (arrow) of bacteria. ×160.

of an organism in pure culture from the liver of diseased quail with which they produced ulcerative enteritis in quail. The organism which they described as a Gram-positive, pleomorphic, aerobic, nonmotile rod. The organism could not be isolated on solid media and on primary culture grew poorly in fluid media. The organism retained its virulence for seven subcultures but the majority of cultures soon lost their virulence.

Bass (1941a) described the isolation of a Gram-negative, anaerobic bacillus from the intestine and liver of infected quail. The medium he used for isolation was thioglycollate modified by the addition of 0.1 per cent agar. Using cultures grown in artificial media, he was able to reproduce the clinical syndrome.

FIG. 17.12 — Seven-day-old culture of quail bacillus in thioglycollate. 9th passage.

Durant and Doll (1941) tested 70 bacterial cultures they had isolated from quail by aerobic and anaerobic culture. None of these cultures reproduced the disease on feeding. On the basis of histological studies and the character of the disease, they concluded the disease was probably of bacterial origin.

Peckham (1959, 1960) reported on the isolation of a Gram-positive, spore-forming rod from the blood, liver and intestine of infected quail. The same organism was isolated from the livers of chickens and turkeys affected with ulcerative enteritis. Most of the isolations were made by yolk sac inoculation of 5–6 day chicken embryos. The intestinal suspension was heated at 70° C. for 10 minutes before embryo inoculation. Some liver suspensions were inoculated directly into embryos without treatment while other suspensions were heated at various temperatures and intervals with the maximum of 80° C. for 20 minutes. Following intraperitoneal injection of quail, 3 isolations were made by inoculating pieces of liver into thioglycollate enriched with 10 per cent horse serum. The fact that the bacillus is a spore former and resistant to heating facilitates isolation. Liver samples that might contain surface contamination can be prepared for embryo or media inoculation by triturating the tissue with a small amount of diluent and then heating at 80° C. for 20 minutes or 100° C. for 3 minutes. Yolk cultures were pathogenic when administered by the oral, intramuscular, and intraperitoneal routes.

CULTURAL CHARACTERISTICS

Growth was established in thioglycollate medium enriched with 3 to 10 per cent horse serum or defibrinated horse blood. After adaptation to thioglycollate, growth occurred as a granular mass in the lower two-thirds of the tube (Fig. 17.12). Following 8 to 10 serial passages in thioglycollate at 48- to 96-hour intervals growth was established on yolk agar, PPLO agar (Difco) with 3 per cent serum fraction, and

5 per cent horse blood agar incubated in 10 per cent CO_2, 63 per cent methane, and 27 per cent air. Initially, light growth occurred in 48 hours as discrete, smooth, raised, white, circular colonies 1 to 2 mm. in size. After adaptation, the growth was not as moist and became confluent. Growth was established on Dorset's egg slants without glycerol and on Loeffler's serum agar slants inoculated with thioglycollate cultures. Direct inoculation of solid media with infected liver did not succeed in establishing growth. The presence of viable organisms in the liver was determined by inoculation of embryos with the same suspension. The use of a staphylococcus streak on blood agar with or without reduced oxygen tension did not enhance growth. The bacillus is Gram-positive in young cultures, in liver smears from birds with necrotic foci in the liver, and in yolk smears from dead embryos. Organisms in old cultures, particularly those grown in artificial media, are decolorized more readily than those from fresh cultures. The bacillus is 3 to 4 microns long and occurs singly as a straight rod or a slightly curved rod with rounded ends. Occasionally, organisms undergoing binary fission are found joined at the ends by a fine strand.

It was noted that variations in morphology occurred with continued passage in artificial media. Coccoid forms, pleomorphic forms, and chains were sometimes seen. However, the organism still retained its rod-shaped morphology even after 14 serial passages in embryos. In embryo cultures, subterminal spores are readily formed. The spores occupy the terminal third of the cell and have a cylindrical form with rounded ends (Fig. 17.13). The spores stain readily with Wirtz spore stain. Smears of quail livers with necrotic foci revealed Gram-positive rods, free spores and rods with subterminal spores. Evidence that the livers with necrotic foci from chickens and turkeys may contain the etiological agent has been demonstrated by infecting quail per os with suspensions of triturated livers (Peckham, 1960).

TRANSMISSION

Under natural conditions the disease is transmitted by ingestion of contaminated droppings. Experimentally, the disease can readily be transmitted by feeding intestinal suspensions to quail.

Peckham (1959, 1960) reported that yolk cultures were pathogenic when adminis-

FIG. 17.13 — Blood smear from a quail with ulcerative enteritis. Note two bacteria, one of which has a subterminal spore. ×2700.
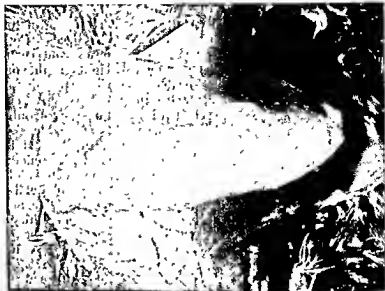
FIG. 17.14 — Large white area of degeneration in the breast muscle of a quail produced by intramuscular inoculation of a yolk culture.

tered to quail by the oral, intramuscular, or intraperitoneal routes. By these methods of inoculation, the earliest deaths occurred 18 hours after inoculation. Usually, intramuscular and intraperitoneal inoculation produced the earliest deaths. Following intramuscular inoculation of the breast muscle, a large white area of degeneration surrounded the site of inoculation, and there was a small amount of clear watery fluid between the muscle layers (Fig. 17.14). After intraperitoneal inoculation a small amount of clear watery fluid was noted in the abdominal cavity. If death occurred early, the intestinal lesions produced by intraperitoneal or intramuscular injection were either a markedly reddened intestinal mucosa or newly formed, acutely inflamed ulcers, and if death was delayed several days, the ulcers became necrotic. The livers had a mottled appearance. Yolk cultures of the bacillus were still infectious for quail after storage for 14 months at 20° C. Cultures in artificial media were nonpathogenic when fed to quail. One culture, serially passed through five transfers in thioglycollate followed by one embryo passage, was pathogenic when fed to quail.

Unsuccessful attempts were made to infect mature pheasants, turkey poults, and chicks by feeding yolk cultures which were pathogenic for quail. Yolk cultures were nonpathogenic when inoculated intraperitoneally into 3-week-old mice. The organism was not pathogenic for guinea pigs when injected into the thigh muscles using the calcium chloride technique described by Smith (1954). Shillinger and Morley (1934) observed that the degree of resistance in quail to infection varied and inconsistent results were often obtained by feeding infectious material to apparently susceptible birds. Infection in some birds appeared to build up an active immunity of considerable duration. They also noted that continued passage from one bird to another under laboratory conditions tended to decrease the virulence of the infection.

CONTROL

Control measures that have been tried include bacterins, serological tests, and prophylactic and therapeutic chemotherapy. Bass (1941b) reported that he immunized quail by two intramuscular injections of a bacterin 5 days apart. The bacterin was prepared from 24–36 hour cul-

tures grown in thioglycollate and killed with 1:10,000 dilution of merthiolate. Birds thus immunized survived the feeding of several hundred times the infectious dose for nonimmune birds.

Peckham (1962) reported the results of four trials in which attempts were made to immunize quail by single and multiple intramuscular injections of heat-attenuated and untreated yolk cultures. Although some trials gave encouraging results, other trials indicated no protection was given by the vaccination procedures.

Morris (1948) described in detail the technique of a complement fixation test for the detection of ulcerative enteritis in quail. He stated that the chronic carrier was one of the most important factors in perpetuating the disease and by the use of the complement fixation test, carriers of the disease could be detected. Comparable results were obtained in tests conducted on birds following artificial and natural infection.

Kirkpatrick et al. (1950, 1952a and b) and Kirkpatrick and Moses (1953) reported that streptomycin administered by injection, water, or feed had a prophylactic and therapeutic value against ulcerative enteritis in quail. He also found that chloromycetin at a level of 500 grams per ton of mash gave complete protection. The majority of quail that survived in groups treated with streptomycin and chloromycetin in the feed remained highly susceptible when re-exposed to infectious material. Streptomycin at a level of 60 grams per ton gave complete protection when medication was started prior to infection. He also found that the administration of streptomycin at a level of one gram per gallon of drinking water gave complete protection when administered prior to or concomitant with artificial infection. After re-exposure of one-half of the survivors in groups treated with streptomycin in the water, Kirkpatrick concluded that there were no marked differences in the development of immunity by variations in dosage of antibiotic, the time when treatment was given,

or the duration of treatment. This is in marked contrast to the results obtained in re-exposure of the survivors of a natural outbreak where it was found that these birds were completely refractory to challenge. Kirkpatrick and Moses (1953) reported on the results of using streptomycin in the water against a natural outbreak of ulcerative enteritis on a game farm. On the first day of treatment the concentration of streptomycin in the water was 5 grams per gallon and 1 gram per gallon for the next 19 days. The untreated birds sustained a 21 per cent mortality and the treated birds 4 per cent.

Peckham and Reynolds (1962) reported on the efficacy of chemotherapeutic drugs in the control of experimental ulcerative enteritis in quail. Their results confirmed those of Kirkpatrick et al. (1952b) and it was found that prophylactic administration of streptomycin at a level of 2 grams per gallon of drinking water for 25 days gave complete protection against experimental exposure. Bacitracin fed at a level of 100 grams per ton of feed also gave complete protection. In one drug trial, quail receiving streptomycin in the water or bacitracin in the feed were completely refractory to challenge after medication was discontinued. However, in another trial two groups receiving bacitracin were 100 per cent susceptible to challenge after medication was discontinued. These observations are in agreement with those of Kirkpatrick and Moses (1953) who also noted marked differences in the susceptibility of quail following medication.

In controlling outbreaks of ulcerative enteritis strict isolation should be maintained between groups of infected and healthy quail. As survivors of an outbreak may be carriers of the disease, they should not be mixed with unexposed birds. The causative organism is extremely resistant and this necessitates thorough cleaning and disinfecting of contaminated pens and equipment. Contaminated yards may remain infectious for long periods.

Outbreaks of coccidiosis in chickens and

turkeys that do not respond to the usual treatment should be carefully examined for ulcerative enteritis. If ulcerative enteritis is present, sulfonamide medication should be discontinued and bacitracin or streptomycin should be given in conjunction with the observance of good management practices.

REFERENCES

Barger, E. H., Park, S. E., and Graham, R.: 1934. A note on so-called quail disease. Jour. Am. Vet. Med. Assn. 84:776.

Bass, C. C.: 1939. Observations on the specific cause and nature of "quail disease" or ulcerative enteritis in quail. Proc. Soc. Exper. Biol. and Med. 42:377.

——: 1941a. Quail disease—some important facts about it. Louisiana Cons. Rev. Summer: 11.

——: 1941b. Specific cause and nature of ulcerative enteritis of quail. Proc. Soc. Exper. Biol. and Med. 46:250.

Bullis, K. L., and Van Roekel, H.: 1944. Uncommon pathological conditions in chickens and turkeys. Cornell Vet. 34:312.

Bump, G., Darrow, R. W., Edminster, F. C., and Crissey, W. F.: 1947. The ruffed grouse. N.Y. State Cons. Dept.

Buss, I. O., Conrad, R. D., and Reilly, J. R.: 1958. Ulcerative enteritis in the pheasant, blue grouse and California quail. Jour. Wildlife Mgt. 22:446.

Churchill, H. M., and Coburn, D. R.: 1945. Sulfonamide drugs in the treatment of ulcerative enteritis of quail. Vet. Med. 40:309.

Durant, A. J. and Doll, E. R. 1941. Ulcerative enteritis in quail. Mo. Agr. Exper. Sta. Res. Bul. 325.

Faddoul, G.: 1958. Annual report of the Waltham Field Station, Waltham, Mass.

Gallagher, B. A.: 1924. Am. Game Prod. Assoc. Bul. April:14.

Clover, J. S.: 1951. Ulcerative enteritis in pigeons. Canad. Jour. Comp. Med. 15:295.

Gray, J. F., Snoeyenbos, G. H., and Reynolds, J. M.: 1954. The hemorrhagic syndrome of chickens. Jour. Am. Vet. Med. Assn. 125:144.

Harris, A. H.: 1961. An outbreak of ulcerative enteritis amongst bobwhite quail (Colinus virginianus), Vet. Rec. Jan. 7:11.

Jungherr, E.: 1955. The bloody tiroi of poultry diseases. Eastern States Cooperator 31:6.

Kirkpatrick, C. M., Moses, H. E., and Baldini, J. T.: 1950. Streptomycin studies in ulcerative enteritis in bobwhite quail 1. Results of oral administration of the drug to manually exposed birds in the fall. Poultry Sci. 29 561.

——, Moses, H. E., and Baldini, J. T.: 1952a. The effects of several antibiotic products in feed on experimental ulcerative enteritis in quail Am. Jour. Vet. Res. 13:99.

——, Moses, H. E., and Baldini, J. T.: 1952b Streptomycin studies in ulcerative enteritis in bobwhite quail II Concentrations of streptomycin in drinking water suppressing the experimental disease. Am. Jour. Vet. Res. 13:102

—— and Moses, H. E.: 1953. The effects of streptomycin against spontaneous quail disease in bobwhites. Jour. Wildlife Mgt. 17 24.

Klein. 1911. The grouse, health and disease. London, cited by Shillinger and Morley.

LeDune, E. K.: 1933. Ulcerative enteritis in ruffed grouse. Vet. Med. 30:394.

Levine, P. P.: 1932. A report on an epidemic disease in ruffed grouse. Trans. 19th Am. Game Conf. 437.

Morely, L. C., and Wetmore, P. W.: 1936 Discovery of the organism of ulcerative enteritis. Proc. No. Am. Wildlife Conf., Senate Comm. Print, 74th Cong. 2nd session, Washington, D.C. 1936, p. 471.

Morris, A. J.: 1948. The use of the complement fixation test in the detection of ulcerative enteritis in quail. Am. Jour. Vet. Res. 9.102.

Morse, G. B.: 1907. Quail disease in the United States. U.S. Dept. Agr. BAI Cir. No. 109.

Peckham, M. C.: 1959. An anaerobe, the cause of ulcerative enteritis, (quail disease). Avian Dis. 3:471.

——: 1960. Further studies on the causative organism of ulcerative enteritis. Avian Dis. 4:449.

——: 1962. Immunization trials against ulcerative enteritis in quail. Ann. Rep. N.Y.S. Vet. Coll. for 1961-62.

——: 1963. Poultry diagnostic accessions. Ann. Rep. N.Y.S. Vet. Coll. for 1962-63.

——, and Reynolds, J.: 1962. The efficacy of chemotherapeutic drugs in the control of experimental ulcerative enteritis. Avian Dis. 6.111.

Pickens, E. N., DeVolt, H. M., and Shillinger, J. E.: 1932. An outbreak of quail disease in bobwhite quail. Maryland Conservationist 9: Spring.

Rosen, M. H., and Bischoff, A. I.: 1949. Field trials of sulfamethazine and sulfaquinoxaline in the treatment of quail ulcerative enteritis. Cornell Vet. 39:195.

Shillinger, J. E., and Coburn, D. R.: 1940. Diseases of game birds Vet. Med. 35:124.

———, and Morley, L. C.: 1934. Studies on ulcerative enteritis in quail. Jour. Am. Vet. Med. Assn. 84:26.

Smith, L. DS.: 1954. Introduction to the Pathogenic Anaerobes. University of Chicago Press, Chicago 37, Illinois, 253 pages.

Stoddard, H. L.: 1931. The Bobwhite Quail—Its Habits, Preservation and Increase. Charles Scribner's Sons, New York, 559 pages.

Witter, J. F.: 1952. Observations on apparent complications of coccidiosis in broiler flocks. Proc. 24th Ann. Conf. Lab. Workers in Pullorum Disease Control, University of Maine, Orono, Maine.

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18

Diseases Caused by Fungi

Advances in the past decade in the control of bacterial and viral diseases of birds have been outstanding. Although much experimental work has been done on the growth of fungi in the chicken egg embryo, very little to no progress has been made in the control of fungous diseases of birds. Fungous diseases are not the most common diseases of birds, yet they are prevalent enough to warrant economic attention.

A bibliography of avian mycosis by Chute, O'Meara, and Barden (1962) lists 709 references to fungi in birds. This bibliography brings out rare cases of reported fungous infections as well as experimental studies relating to birds and chicken egg embryos.

Aspergillosis

Aspergillosis has been observed in many birds and mammals. Frequent reference is made to the relationship of the disease in man to occupation, particularly in the so-called *gaveurs des pigeons* (pigeon feeders).

Occurrence. Aspergillosis is encountered in poultry in two main forms. Acute outbreaks in which there is a high morbidity and a high mortality may occur particularly in young birds. In adults especially, an occasional bird in a flock or aviary may become affected while the other birds remain healthy. The numerous reports in the literature suggest that nearly all species of birds may be affected. The incidence of the disease is not great, however, as evidenced by reports from diagnostic laboratories.

Etiology. It is generally agreed that *Aspergillus fumigatus* Fresenius is the most pathogenic and the most frequently encountered in disease processes due to aspergilli. The spores are widely distributed in nature, and birds frequently come in contact with them through con-

[494]
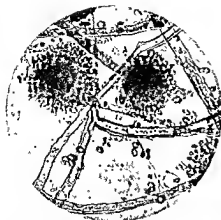
FIG. 18.1 — *Aspergillus fumigatus*. ×250. (From Nowak, *Documenta Microbiologica*, courtesy Gustav Fischer.)

taminated feed or litter. The fungus grows quite readily on the ordinary laboratory culture media at room temperature, at 37° C., and higher. Czapek's solution agar or Sabouraud's agar may be used. The colonies are green to bluish-green at first and darken with age so as to appear almost black. The colonies vary from velvety to floccose. The conidiophores are short, up to 300μ long by 2 to 8μ in diameter, the vesicles are apical flask-shaped up to 20 to 30μ in diameter, the sterigmata are 6 to 8μ by 2 to 3μ, and the conidia are globose 2.5 to 3μ in diameter, in chains forming solid columns up to 400μ by 50μ (Fig. 18.1) (Thom and Church, 1926).

Leber, according to van Heelsbergen (1929) and Lucet (1897), succeeded in isolating toxins from cultures of *Aspergillus fumigatus*. Ceni and Besta (1902) were able to extract toxic materials from spores. A toxin reported by Bodin and Gautier (1906) was similar to bacterial toxins and produced clonicotonic convulsions, paralytic symptoms, and finally death. A toxin obtained by Henrici (1939) was toxic for rabbits, guinea pigs, mice, and chickens. This toxin was hemotoxic, neurotoxic, and histotoxic. Rabbits and dogs are very susceptible to *Aspergillus* toxin. Pigeons, however, which are very susceptible to spontaneous infection are very resistant to injected toxin.

Forgacs and Carll (1955) selected strains of *Aspergillus, Penicillium,* and *Alternaria*, isolated from samples of feed associated with outbreaks of a hemorrhagic disease in poultry, and grew the cultures on grain which was fed to day-old chicks. Subsequently the chicks developed hemorrhages of the muscles, lungs, heart, gastrointestinal tract, and liver. Twenty chicks, 6 weeks of age, were given feed on which the species of *Alternaria* had been cultured alone; they died and the necropsy revealed hemorrhages throughout the carcass.

The fungous flora of young broiler chicks up to 13 weeks of age has been extensively studied by Chute *et al.* (1956). These workers observed that *A. fumigatus* may be found frequently and is not always pathogenic. The following genera were found in the lungs and air sacs: *Aspergillus, Penicillium, Paecilomyces, Cephalosporium, Trichoderma, Scopulariopsis,* and *Mucor.*

Chute and O'Meara (1958) found the air sac inoculation route with spores to be a rapid and efficient method of screening fungi for pathogenicity in chickens. The following cultures isolated from chickens were used in experimental air sac infections and revealed both spores and mycelia in the tissue: *Paecilomyces varioti, Penicillium roqueforti, Penicillium brevi-compactum, Aspergillus glaucus* groups, *Trichoderma* spp., *Trichoderma koningi, Penicillium oxalicum, Aspergillus fumigatus, Alternaria* spp., *Penicillium islandicum, Stemphylium* spp., and *Penicillium cyclopium.*

Vigorous, healthy birds apparently can withstand considerable exposure to *Aspergillus* spores occurring under natural conditions. Inhalation of a considerable number of spores, as may occur when the litter or feed are heavily contaminated, may result in infection. The occasional bird which becomes infected in a flock which is otherwise healthy may do so because of lowered resistance or severe individual exposure. Aspergillosis can readily be produced experimentally by intrathoracic injection in chickens and

pigeons. Schutz (1881), Bollinger, cited by van Heelsbergen (1929), and others observed that infection of the lungs was established following inhalation of spores. Walker (1915) reported that 5- to 7-day-old ostriches succumbed in 2 to 8 days to aspergillosis in the lungs and air sacs if spores were blown into the trachea. Intravenous inoculation resulted in pulmonary and hepatic aspergillosis. Young ostriches also developed the disease when kept on straw which had been artificially contaminated. Durant and Tucker (1935) produced the disease in a poult by feeding mash from which A. fumigatus was isolated.

In recent years several reports have been made relative to aspergillus eye infections. Reis (1940) described a keratitis in chicks caused by A. fumigatus. He described the pathology and stated usually only one eye was involved. Moore (1950) occurred in five widely separated flocks of young poults and in three breeding flocks (Fig. 18.2).

Systemic aspergillosis in poults has been reported by Witter and Chute (1952). Chute et al. (1955) also reported a systemic aspergillosis infection in 5-week-old cockerels which had been caponized. The authors considered that this resulted from a caponizing infection.

Aspergillus glaucus and A. niger may

FIG. 18.2 — Plaque from the eye of a chick with eye aspergillosis. X 2. (Chute, University of Maine)

be encountered in some cases, particularly in cutaneous lesions. Lahaye (1928) discusses cutaneous aspergillosis in pigeons. Ainsworth and Rewell (1949) reported the isolation of 45 pure cultures of *A. fumigatus*, 3 of *A. flavus*, and 1 of *A. nidulans* from 78 cases of aspergillosis in captive wild birds. Jungherr and Gifford (1944) found fungal hyphae in the cerebellum of a poult which had exhibited nervous symptoms. In another outbreak in poults showing pneumomycosis, these workers recovered *A. fumigatus*, *A. niger*, and *Paecilomyces varioti* from the internal organs. The latter was isolated also from the brain of one poult, but since fungal hyphae could not be demonstrated and the culture proved nonpathogenic, it was concluded that the symptoms and brain lesions had a toxigenic base. Bullis (1950) recovered *A. fumigatus* from the cerebrums of poults which showed incoordination and later (unpublished) *Diplococcium* sp. from the cerebrums of similar poults. *Mucor* sp. and *Penicillium* sp. and other fungi may be encountered in pulmonary mycosis, particularly in mixed infections (Baker *et al.*, 1934; Thompson and Fabian, 1932).

A case of egg-borne aspergillosis was reported by Eggert and Barnhart (1953). They suggested the fungus had penetrated through the eggshell during incubation and the recently hatched chicks were infected. Another case which was shown to be hatchery-borne was reported by Clark *et al.* (1954). From 21 ranches where 210,000 chicks were involved, there was mortality from 1 to 10 per cent. The infection could not be traced to the hatching eggs but was readily found in the incubators, hatchers, incubator rooms, and intake ducts. Symptoms and lesions were noted in some day-old chicks but generally classical lesions were observed in chicks 5 days of age.

O'Meara and Chute (1959) produced aspergillosis experimentally in hatching chicks. Chicks in the process of hatching and up to two days of age were easily infected with *A. fumigatus* spores by con-
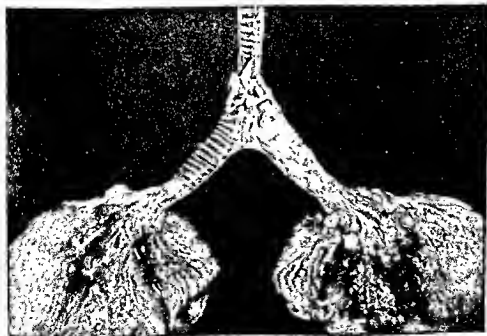
FIG. 18.3 — Aspergillosis involving syrinx. (Bullis, University of Massachusetts)

taminating the forced draft incubator with wheat which had been seeded with *A. fumigatus* Chicks older than three days of age were resistant to infection.

Chute and O'Meara (1961) reported some unusual cases of avian mycosis. Two cases of fungous tracheitis were observed and in each case the mycelial growth extended through the cartilage.

Lesions. The lesions depend considerably on the site of infection. Either localization or generalization may be observed. Individual lesions may be observed, for example, in the syrinx (Fig. 18.3) or in a single air sac. The lungs are most frequently involved. Pulmonary lesions vary from miliary nodules up to larger nodules (Fig. 18.4). In some cases there may be localized hepatization, and in others grossly visible mycelial masses may be present in the air passages and bronchi There may be generalized involvement of the air sacs. Occasionally, a circular disc-shaped necrotic mass with a concave surface, loosely attached to which there is a circular more or less flat or convex plaque, may be observed. Various manifestations of the disease have been described. Lange (1914) recorded nodules in the lungs, the thoracic, and the abdominal cavities of chickens, ducks, geese, and pigeons. These nodules varied from pinhead or millet seed size up to the size of a pea. They were yellow in color, of an elastically soft or cartilaginous consistency, and homogeneously caseous Individual nodules

were noted on the intestinal serosa and in the parenchyma of the liver in a goose.

The nodules in the lungs of a turkey observed by Schlegel (1915) were pinhead to lentil size and were surrounded by an infiltrated or hemorrhagic corona with considerable hepatization. There were also grayish-yellow, fibrinopurulent disc- or plate-shaped masses of exudate 2 to 5 mm. thick on the pleura. Inflammation and detrital masses were present in the bronchi. The anterior thoracic, the axillary, and the cervical air sacs contained yellow caseous flat discs and masses consisting of inflammatory exudate and mycelia. The left lower, upper posterior thoracic, and left abdominal air sacs were greatly distended. The walls of these air sacs were thickened and covered with a furlike growth of mold. Adjacent to the air sacs there were lentil-sized, knob-shaped, and concentrically layered, turbid yellow, solid nodules composed of fibrin and mycelia. There were about 200 cc. of a reddish, turbid fluid in the abdominal cavity.

There were no circumscribed yellowish foci in the outbreak in chicks reported by Savage and Isa (1933). There was a diffuse grayish-yellow infiltration in the lungs with about one-third of each lung involved. The mortality was 90 per cent in this outbreak.

In pneumomycosis in a flamingo described by Mohler and Buckley (1904), the lungs were filled with nodules, and
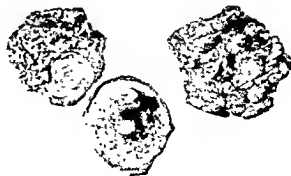
FIG. 18.4 — Aspergillosis nodules in lungs and plaquelike formations on the serous membranes. (Bullis, University of Massachusetts)

the mucosa of the bronchi was covered with membranous masses that consisted primarily of the fungous mycelium.

Archibald (1913) found gray, round colonies of the fungus in the bronchioles in an ostrich, whereas in a case described by Jowett (1913) the lungs were covered with miliary foci.

Lahaye (1928) states that *Aspergillus glaucus* may be the cause of a disease of the skin in pigeons, particularly in young birds. Any part of the body may be affected with yellow scaly spots. The feathers in the affected areas are dry and easily broken.

Durant and Tucker (1935) observed yellowish-white nodules up to 8 × 5 mm. in the lungs of wild turkey poults being reared in captivity. The hyphae of the fungus also penetrated the tissue of the lung, and there was involvement of the adjacent air sacs.

In canaries observed by de Jong (1912) there were small whitish-yellow, crusty coatings on the tongue, palate, aditus laryngis, and in the trachea and syrinx. Caseous foci in the lungs and caseous coatings on the pleura and peritoneum were also observed.

The histological picture as described by Nieberle (1923) consists of focal pneumonia, multiple necrosis, and nodular formations which resemble tubercles. The diffuse pneumonic foci are indicative of the fibrinous or catarrhal pneumonia. The alveoli, bronchioles, and bronchi are filled with mucus, stained fibrin, nuclear fragments, detritus, leucocytic and inflammatory cells, and mycelia. The mycelia penetrate the walls, and the surrounding pulmonary parenchyma shows an exudative cellular inflammation or necrosis. The tuberclelike nodules show in the center a radiating turf of hyphae surrounded by a reactive inflammatory wall which resembles granulation tissue. Foreign body giant cells are frequently observed. The fruiting organs (conidiophores, sterigmata, conidia) occur more frequently in the air sacs.

Symptoms. Dyspnea, gasping, and ac-

celerated breathing may be present. When these symptoms are associated with other respiratory diseases such as infectious bronchitis and infectious laryngotracheitis, they are usually accompanied by gurgling and rattling noises, whereas in aspergillosis there usually is no sound. Guberlet (1923) ascribed somnolence, inappetence, emaciation, increased thirst, and pyrexia to aspergillosis. Cases under his observation emaciated rapidly and showed a diarrhea in the later stages. Dysphagia was noted in cases in which the esophageal mucosa was involved. Mortality was as high as 50 per cent in confined birds on some farms, whereas birds running out of doors were more resistant and escaped infection entirely on other farms. According to van Heelsbergen (1929) some investigators have reported serous excretions from the nasal and ocular mucosa. Extreme dyspnea was recorded by de Jong (1912) in canaries. In an outbreak in wild turkey poults reared in captivity, described by Durant and Tucker (1935), mortality began at 5 days, reached a peak at 15 days, and subsided at three weeks of age. Some affected poults died in convulsions within 24 hours. In two lots of poults 200 survived out of 785. Gauger (1941) reported an outbreak in adult chickens in which about 10 per cent of the flock was affected with symptoms not unlike those shown by birds affected with laryngotracheitis and in which there was no abnormal mortality, but the egg production was temporarily lowered. Reis (1940), cited by Hudson, and Hudson (1947) have reported infection of the eyes in chicks two to five weeks of age. Infection in Reis's cases originated in sawdust litter and in Hudson's cases in bagasse litter. The outbreaks were characterized by the formation of a yellow cheesy pellet beneath the nictitating membrane which caused the lids to bulge (Fig 18.2). There was some central ulceration of the cornea in the older chicks.

Diagnosis. The fungus can be demonstrated by cultural methods if it cannot be demonstrated in fresh microscopic

FIG. 18.5 – Granuloma of lung from chick infected with A. fumigatus. (Chute, Univ. of Maine)
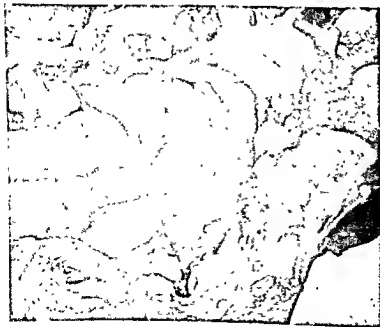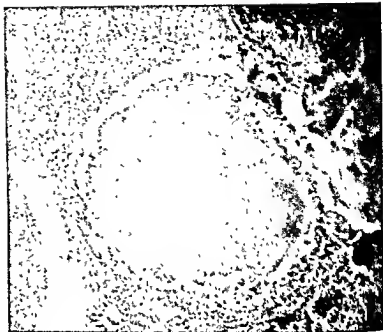
FIG. 18.6 – Viscera and musculature with plaques from a chicken experimentally infected in the air sac with A. fumigatus. (Chute, Univ. of Maine)

preparations. Occasionally, masses of the fungus are visible to the naked eye in the air passages of the lungs (Fig. 18.5), in the air sacs (Fig. 18.6), or in the abdominal cavity. The typical fruiting heads may be readily demonstrated in such lesions. Demonstration of the fungus by direct examination may be impossible in the small caseous nodules seen particularly in the lungs.

Prophylaxis and treatment. The avoidance of moldy litter or feed serves to prevent outbreaks of aspergillosis. An examination of the premises or materials used

for feed or litter will usually reveal the source of the infection.

Treatment of affected individuals is usually considered useless. They should be sacrificed and the offending infective material removed. Lahaye (1928) reported favorable results in the treatment of aspergillosis of the skin in pigeons by the use of $HgCl_2$ solution 1:500. The surface of the body was moistened or the birds dipped into the solution, following which they were rinsed in lukewarm water and dried.

REFERENCES

Ainsworth, G. C., and Rewell, R. E.: 1949. The incidence of aspergillosis in captive wild birds. Jour. Comp. Path. and Therap. 59:213.

Archibald, R. G.: 1913. Aspergillosis in the Sudan ostrich. Jour. Comp. Path. and Therap. 26:171.

Baker, A. Z., Courtenay-Dunn, J., and Wright, M. D.: 1934. Observations on fungal pneumonia in the domestic fowl. Vet. Jour. 90:585.

Bodin, E., and Gautier, L.: 1906. Note sur une toxine produite par l'Aspergillus fumigatus. Ann de l'Inst. Past. 20:209.

Bullis, R. L.: 1950. Poultry Disease Control Service. Mass. Agr. Exper. Sta. Ann. Rep., Bul. 459.85.

Ceni, C., and Besta, C.: 1902. Ueber die Toxine von Aspergillus fumigatus und A. flavescens und deren Beziehungen zur Pellagra. Zentralbl. f. Allg. Path. und Path. Anat. 13:930.

Chute, H. L., and O'Meara, D. C.: 1958. Experimental fungous infections in chickens. Avian Dis. 2:154.

——, and O'Meara, D. C.: 1961. Diagnosis of unusual cases of avian mycosis. Canad Vet. Jour. 2:385.

——, O'Meara, D. C., and Barden, E. S.: 1962. A bibliography of avian mycosis (partially annotated). Dept. of Anim. Path., Univ. of Maine. Orono, Maine. Misc. Publ. No. 655.

——, O'Meara, D. C., Tresner, H. D., and Lacombe, E.: 1956. The fungous flora of chickens with infections of the respiratory tract. Am. Jour. Vet. Res. 17:763.

——, Witter, J. F., Rountree, J. L., and O'Meara, D. C. 1955. The pathology of a fungous infection associated with a caponizing injury. Jour. Am. Vet. Med. Assn. 127:207.

Clark, D. S., Jones, E. E., Crowl, W. B., and Ross, F. K.: 1954. Aspergillosis in newly hatched chicks. Jour. Am. Vet. Med. Assn. 124:116.

de Jong, D. A.: 1912. Aspergillosis der Kanarienvögel. Zentralbl. f. Bakt. 1. Orig. 66.590.

Durant, A. J., and Tucker, C. M.: 1935. Aspergillosis of wild turkeys reared in captivity. Jour. Am. Vet. Med. Assn. 86:781.

Eggert, M. J., and Barnhart, J. V.: 1953. A case of egg-borne aspergillosis. Am. Jour. Vet. Med. Assn., 122:225.

Forgacs, J., and Carll, W. T.: 1955. Preliminary mycotoxic studies on hemorrhagic disease in poultry. Vet. Med. 50.172.

Gauger, H. C.: 1941. Aspergillus fumigatus infection in adult chickens. Poultry Sci. 20.445.

Guberlet, J. E.: 1923. An epizootic of aspergillosis in chickens. Jour. Am. Vet. Med. Assn. 63.612.

Henrici, A. T.: 1939. An endotoxin from Aspergillus fumigatus. Jour. Immunol. 36:319.

Hudson, C. B.: 1947. Aspergillus fumigatus infection in the eye of baby chicks. Poultry Sci 26:192.

Jowett, W.: 1913. Pulmonary mycosis in the ostrich. Jour. Comp. Path. and Therap. 26:253.

Jungherr, E., and Gifford, R.: 1944. Three hitherto unreported turkey diseases in Connecticut, erysipelas, hexamitiasis, mycotic encephalomalacia. Cornell Vet. 34:214.

Lahaye, J.: 1928. Maladies des pigeons et des poules, des oiseaux de basse-cour et de volière. Renouchamps: Stemmetz-Haenen.

Lange, W.: 1914. Schimmelpilzerkrankungen beim Geflügel. Deutsch. tierärztl. Wochenschr 22:642.

Lucet, A.: 1897. Experimental and clinical study of Aspergillus fumigatus. Vet. Jour. 44:215–17; 285–88; 392–91; 45:226–31; 301–4. (Translated from Bul. de la Soc. Centrale de Méd. Vet.)

Mohler, J. R., and Buckley, J. S: 1904. Pulmonary mycosis of birds with report of a case in a flamingo. Bur. Anim. Ind., U.S.D.A., Circ. 58.122.

Moore, Earl N.: 1950. Ophthalmic aspergillosis in turkeys. Proc. 22nd Ann. Meet. of Northeastern Conf. of Lab. Workers in Pullorum Control. June 20–21, 1950

Nieberle, R · 1923 Die Lungenaspergillose. In Joest, E.: Spez. path. Anat. der Haustiere. R Schoetz, Berlin 3.804.

O'Meara, D C., and Chute, H. L.: 1959. Aspergillosis experimentally produced in hatching chicks Avian Dis 3.404.

Reis, J: 1910 Queratomicose Aspergillica Epizoótica em Pintos. Arq. Inst., São Paulo, Brazil, Brazil, Vol 11, Artigo 48:437.

Savage, A., and Isa, J. M.. 1933. A note on mycotic pneumonia of chickens. Scient. Agr. 13,541.

Schlegel, M: 1915. Schimmelpilzerkrankung (Aspergillose) in den Lungen bei Tieren. Berliner tierärztl. Wochenschr. 31.25.

Schütz 1884 Eindringen von Pilzsporen in die Atmungswege und die dadurch bedingten Erkrankungen der Lungen und über den Pilz des Hühnergrindes. Mittheil. a. d. Kaiserl Gesundheitsamt 2:208

Theen, C., and Church, Margaret B., 1926 The Aspergilli. Williams and Wilkins Co., Baltimore. 272 pp

Thompson, W. W., and Taban, F. W.: 1932 Molds in respiratory tract of chickens. Jour. Am Vet Med. Assn 80 921

van Heelsbergen, T 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart. Pp 312–22

Walter, J · 1915. Aspergillosis in the ostrich chick. Union of South Africa, Dept. Agr. Ann. Rpts. Dir Vet Res. 3–4, 535–74.

Witter, J F, and Chute, H L · 1952. Aspergillosis in turkeys. Jour. Am. Vet. Med. Assn. 121,587.

Favus

Favus is a chronic dermatomycosis affecting chickens, occasionally turkeys and some other birds, animals, and man. In the fowl, the comb is almost always attacked, but other portions of the head may be affected, and in severe cases the disease spreads to the feathered portions of the body.

Occurrence. Favus occurs only infrequently in the United States. Possibly this is due to the lesser number of the heavier Asiatic breeds which are reported to be more susceptible The disease is reported to be quite common in France.

Etiology. The causative fungus in the fowl is *Trichophyton megnini (Achorion gallinae)*. Cultivation on Sabouraud's glucose agar is satisfactory. It is sometimes of assistance to cover the inoculum with absolute alcohol and evaporate the alcohol to destroy the contaminating bacteria. Cultures grow slowly. Colonies develop as small round discs which are white and velvety and have small central cups and radial grooves. A reddish pigment varying from rose or strawberry red to a deep raspberry diffuses through the medium. Microscopically, the branched mycelium is twisted, the septa are irregularly spaced, the spores are in clusters, and there are nodular organs and fuseaux. Typical lesions may be produced by inoculation of the scarified comb. Mice, rabbits, or

guinea pigs may also be inoculated, although the lesions are not so typical in the guinea pigs (Dodge, 1935; Jacobson, 1932). Torres and Georg (1956) reported a case of *Trichophyton gallinae* in the scalp of a 4-year-old Puerto Rican girl. Experimental infections were produced in several chickens and guinea pigs.

Symptoms and lesions. Lesions usually develop first on the comb. As the fungus spreads, white spots develop, the surface of which scales off, and the comb may appear as though sprinkled with flour (Fig. 18.7). Young birds with well developed combs are most likely to be affected. The wattles and unfeathered por-

FIG. 18.7—Favus of comb and wattles. (Bullis, University of Massachusetts)

tions of the head may be affected. As the disease progresses, the scaly deposits become thicker and form a wrinkled crust. Spontaneous recovery is reported in some cases. In other cases the fungus spreads to feathered portions of the body. The feathers fall out in patches. The skin becomes thickened in the affected areas and covered with scales and crusts, especially around the feather follicles. A moldy odor may be detected. Matruchot and Dassonville (1899) reported the appearance of favus simultaneously on the feathered and nonfeathered portions of the body. Symptoms were not extensive in the cases observed by Sabouraud et al. (1909). Schlegel (1909) reported depression, weakness, emaciation, anemia, cachexia, and icterus in affected chickens. In some birds, in addition to the external lesions, there were necrotic foci, nodules, and yellowish caseous deposits on the mucosa of the upper respiratory and digestive tracts. Occasionally, the bronchi and lungs were affected and necrotic caseous inflammation was observed in the crop and small intestine. The favus fungus could be demonstrated microscopically in these lesions. The fungus spreads slowly from bird to bird by direct contact and by the scales which become detached from affected individuals and contaminate the premises.

Diagnosis. The characteristics of the gross lesions may be sufficient for diagnosis. If this is inconclusive, the fungus can be checked microscopically and culturally. Transmission of the disease to laboratory birds or animals or a study of the contagious nature of the disease in the flock may be helpful.

Prophylaxis and treatment. Care should be exercised in the addition of new birds to the flock. Infected houses should be cleaned and disinfected. Badly affected birds should be sacrificed. Mildly affected birds should be segregated, and treatment can be tried if desired. The majority of mildly affected birds will recover without treatment. Several individuals have been observed in which various treatments were used on one side of the head and the opposite side was left untreated, and recovery was similar on each side. Van Heelsbergen (1929) suggests the following remedies: iodine and glycerine (tinct. iodine 1.0, glycerine 6.0), green soap, and 5 per cent phenol solution, or bichloride of mercury (1:500), the latter to be used particularly on the body. Beach and Halpin (1918) found an ointment of formaldehyde and vaseline to be effective. This is prepared by melting vaseline in a jar in a water bath. Five per cent by weight of commercial formalin is added, the cover tightened, and the mixture shaken until the vaseline has solidified. One or two applications well rubbed into the lesions usually suffice. Riedel (1950) observed recovery in a group of artificially infected chickens within 20 days following a single application of a 2 per cent mixture of quaternary ammonium compounds consisting of equal parts of alkyl-dimethyl-benzyl-ammonium chloride and a l k y l-dimethyl-dichlor-benzol-ammonium chloride.

REFERENCES

Beach, B. A., and Halpin, J. G.: 1918. Observations on an outbreak of favus. Jour. Agr. Res 15.415.

Dodge, C. W.: 1935. Medical Mycology. C. V. Mosby Co., St. Louis. Pp 551-55

Jacobson, H. P.: 1932. Fungous Diseases. Charles C Thomas, Springfield, Illinois. Pp. 52-55.

Matruchot, L., and Dassonville, C.: 1899. Recherches expérimentales sur une dermatomycose des poules et sur son parasite. Rev. Gén. de Bot. 11-429.

Riedel, B. B.: 1950. Favus and its treatment with a quaternary ammonium compound Poultry Sci. 29.741.

Sabouraud, R., Sutt, A., and Sufran, F.: 1909. La "cette blanche" (favus) de la poule et son parasite Rév. Vét. Méd. 34 601, 672.

Schlegel, M.: 1909. Favuskrankheit (Flühnergrind). Berlin, tierärzth. Wochenschr. 25 689.

Torres, L., and Georg, L. K. 1956. A human case of Trichophyton gallinae infection contracted from chickens Amer. Pub. Health Assoc. J. 64 Meet., p. 64 New York

van Heelsbergen, T. 1929 Handbuch der Geflugelkrankheiten und der Geflugelzucht Ferdinand Enke, Stuttgart Pp. 322-27

Thrush (Mycosis of the Digestive Tract)

Stomatitis oidica, muguet, soor, moniliasis, oidiomycosis, and sour crop are other terms applied to mycotic affections of the digestive tract.

Occurrence. Mycosis of the digestive tract probably occurs rather frequently, but in many cases it does not appear to be of sufficient significance to be considered seriously. Numerous general discussions of poultry diseases fail to mention this disorder, and the paucity of diagnoses in reports from diagnostic laboratories suggests that it may not be of great consequence. However, serious outbreaks have been reported in many species of birds Animals and man are also affected Thrush has been observed in chickens, pigeons, geese, turkeys, pheasants, ruffed grouse, and quail.

Etiology. The etiological significance of yeastlike fungi in affections of the digestive tract of man was recognized by Langenbeck in 1839. Questions relating to the validity of species described and their generic nomenclature have retarded a proper understanding of this type of disease. Jungherr (1935b, 1934) found *Monilia albicans*, *Monilia krusei*, and *Oidium pullorum* n s. to be associated with cases of thrush, but considered that *M. krusei* was not of etiological significance. *Mucor* sp and aspergilli were also found in association with some cases. Hinshaw (1933) reported that *M. albicans* was found in most cases of thrush in turkeys and chickens which came to his attention. Both investigators considered that the mycotic infections are apt to be associated with unhygienic surroundings and perhaps secondary to other debilitating conditions Eberth (1858) and Schlegel (1912) identified organisms observed by them as *Oidium albicans*.

The studies of Worley and Stovall (1937), Benham (1931), Martin and associates (1937), and others indicate the complexity of the problem. Stovall (1939) pointed out a means of improving the present uncertain status. He suggested a

specific set of environmental conditions under which the biological characteristics of the organism were constant and could be demonstrated. Jungherr's (1934) characterization is as follows: "*Monilia albicans*: It is of widespread occurrence in gallinaceous birds, pathogenic to birds and also to rabbits on intravenous injection, and is indistinguishable from strains isolated from human sources. On Sabouraud's agar it produces a whitish, creamy, high-convex colony after incubation for 24 to 48 hours at 37°C. Young cultures consist of oval budding yeast cells, about 5½ by 3½μ in dimension. Older cultures show septate hyphae and occasionally spherical, swollen cells with thickened membrane, the so-called chlamydospores. In Dunham's peptone water containing 1 per cent fermentable substance and 1 per cent Andrade's indicator, the organism produces acid and gas in dextrose, levulose, maltose, and mannose; slight acid in galactose and sucrose; and does not attack dextrin (variable according to brand), inulin, lactose, and raffinose. Gelatin stab cultures show short, villous to arborescent outgrowths without liquefaction of the medium."

The term "medical monilias" is frequently used in connection with the generic term Monilia since the term Monilia is also used for a separate group of fungi. Most workers have accepted the decision of an informal group meeting at the Third International Microbiological Congress in 1939 and use Candida as a generic name to replace the familiar but invalid *Monilia* (Skinner, 1947). *Candida albicans* is the most frequently isolated etiological agent associated with the disturbance commonly referred to as moniliasis

Symptoms and lesions. The symptoms are not particularly characteristic. Affected chicks show unsatisfactory growth, a stunted appearance, listlessness, and roughness of the feathers. Lesions occur most frequently in the crop (Fig. 18.8) and consist of a thickening of the mucosa with whitish, circular, raised ulcer for-

mations, the surfaces of which tend to scale off. Pseudomembranous patches and easily removed necrotic material over the mucosa are not uncommon. The mouth and esophagus may show ulcerlike patches. When the proventriculus is involved, it is swollen, the serosa has a glossy appearance, and the mucosa is hemorrhagic and may be covered with a catarrhal or necrotic exudate. Histologically, Jungherr (1933a) reports the crops "showed extensive destruction of the stratified epithelium deep in the Malpighian layer and quite often walled-off ulcers or extensive diptheroid to diphtheritic membranes. The lesions were characterized by the absence of inflammatory reaction." Periportal focal necrosis in the liver in some cases suggested a toxic action upon the system.

The frequent association of mycosis of the digestive tract with other debilitating conditions such as gizzard erosions and intestinal coccidiosis must be considered. Gizzard erosions as such probably are not directly related to thrush. Likewise, the thickened intestine with watery contents frequently noted in cases of thrush is probably due to coccidiosis or other protozoan infections.

In the case of thrush reported by Eberth (1858), the esophagus, crop (Figs. 18.8 and 18.9), and proventriculus showed an ulcerated and scaly condition. The spores and hyphae of what he termed Oidium albicans could be readily demonstrated in the lesions. The proventriculus was the principal organ involved in the cases observed by Schlegel (1912). The mouth, pharynx, and crop were, however, involved in some cases. Schlegel (1921) also observed the disease in geese. Diphtheroid lesions were noted in the proventriculus and small intestine. Abscess formations were present under pulpy, soft, grayish-white to brown-
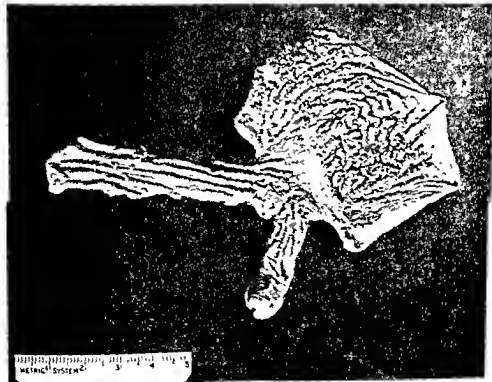
FIG. 18.8 — Moniliasis (candidiasis) in crop of turkey. (Bullis, University of Massachusetts)
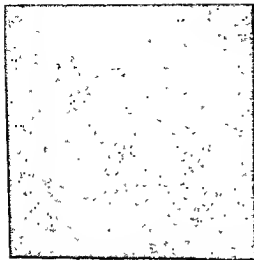
FIG. 18.9 — Mycelia in stratified squamous epithelium of crop. Gridley stain. (Chute, Univ. of Maine)

ish-red necrotic masses. Hinshaw (1933) reported thrush in twelve flocks of turkeys, and the lesions were similar to those noted in chickens. Blaxland and Fincham (1950) studied five serious outbreaks in young turkeys. Their observations supported previous conclusions that moniliasis is likely to be associated with unhygienic surroundings and other debilitating conditions, but spread of infection appeared definite in many instances. Zürn (1882) and Klee (1899) described the disease in pigeons. Lahaye (1928) pointed out the similarity between thrush and pox in the pigeon. He demonstrated pox virus in many cases suspected of being thrush.

Diagnosis. Observation of the characteristic proliferative, relatively noninflammatory lesions, together with resultant heavy growth on primary cultures, serves to diagnose thrush. Because of the possibility of cultivation of *C. albicans* from apparently normal tissues, an original heavy growth is considered essential for diagnosis. The recognition of spores and more especially hyphae in fresh smear preparations is attended with some difficulty. Miliary abscesses are produced in the kidneys of rabbits injected intravenously (Benham, 1931).

Underwood (1955) described an instrument known as a McCarthy's foroblique panendoscope which was used to diagnose experimental crop moniliasis. This instrument was equipped with a viewing lens and an independent light source. Birds were starved for 12 hours in order to empty the crop to allow a clear view of the mucosa. A normal crop appeared to be light pink with a glistening smooth surface having numerous shallow convolutions, whereas a fungus-infected crop showed severe corrugations or mild whitish streaks, erosions, or diphtheritic formations and a deep red surrounding mucosa.

Course. Young birds are more susceptible to mycosis of the digestive tract than are older birds. Thus as an infected group of birds grow older they tend to overcome the infection. Jungherr (1933a) observed an outbreak in which the losses amounted to 10,000 chicks out of 50,000 that were less than 60 days of age. He also reported (1934) that turkeys under four weeks of age succumbed rapidly to infection, but that outbreaks in turkeys three months of age resulted in a high percentage of recoveries.

Prophylaxis and treatment. Since mycosis of the digestive tract is apt to be related to unhygienic, unsanitary, overcrowded conditions, these factors should not be allowed to exist, or should be corrected. Jungherr (1933b) found that denatured alcohol and coal-tar derivatives were ineffective as disinfectants and suggested that iodine preparations be used. As a treatment he recommends that following an Epsom salt flush, one level teaspoonful of powdered blue stone (copper sulfate) be added to each 2 gallons of drinking water in nonmetal containers every other day during one week. Hinshaw recommends that a 1:2,000 solution of copper sulfate for turkeys be used as the sole source of drinking water during the course of the outbreak. Affected birds should be segregated. Lesions in the mouth can be treated by local application of a suitable antiseptic. The appearance of the disease in very young chicks sug-

gests the surface of the egg as a source of infection. Such a possibility could be removed by dipping the eggs in an iodine preparation prior to incubation.

Underwood et al. (1956), in experimental moniliasis produced in chicks and poults, found copper sulfate was ineffective for treating or preventing the disease.

Nystatin has been studied by Gentry et al. (1960) and by Kahn and Weisblatt (1963). One group reported that 220 mg.

Nystatin per kg. of diet fed was effective in eliminating moniliasis in a flock of turkeys. The other group found in experimental infections with C. albicans in both chickens and turkeys that crop score values appeared to be significantly reduced in the group fed the lowest level of Nystatin (11 mg/kg). The highest level of Nystatin (110 mg/kg) fed showed a very significant protection against mycotic infection.

REFERENCES

Benham, R. W.: 1931. Certain Monilia parasitic on man. Jour. Infect. Dis. 49:183.

Blaxland, J. D., and Fincham, I. H.: 1950. Mycosis of the crop (moniliasis) in poultry. Brit. Vet. Jour. 106:221.

Eberth, J.: 1858. Einige Beobachtungen von pflanzlichen Parasiten bei Thieren. Arch. f. path. Anat. u. Physiol. 15:522.

Gentry, R. F., Bubash, G. R., and Chute, H. L.: 1960. Candida albicans in turkeys. Poultry Sci. 39:1282.

Hinshaw, W. R.: 1933. Moniliasis (thrush) in turkeys and chickens. Proc. Fifth World's Poultry Cong., Paper 97, p. 190.

Jungherr, E.: 1933a. Observations on a severe outbreak of mycosis in chicks. Jour. Agr. Res. 46:169.

——: 1933b. Studies of yeast-like fungi from gallinaceous birds. Storrs Agr. Exper. Sta. Bul. 188.

——: 1934. Mycosis in fowl caused by yeast-like fungi. Jour. Am. Vet. Med. Assn. 84:1500.

Kahn, S. G., and Weisblatt, H.: 1963. A comparison of Nystatin and copper sulfate in experimental moniliasis of chickens and turkeys. Avian Dis. 7:3, 301.

Klee, R.: 1809. Vergiftungen bei Geflügel. Jahresber. Vet. Med. 10.256.

Lahaye, J.: 1928. Maladies des pigeons et des poules, des oiseaux de basse cour et de volière. Remouchamps: Steinmetz-Haenen.

Martin, D. S., Jones, C. P., Yao, K. F., and Lee, L. E., Jr.: 1937. A practical classification of the Monilias. Jour. Bact. 34:99.

Schlegel, M.: 1912. Soorkrankheit bei Hühnern. München. tierärtl. Wochenschr. 56:63.

——: 1921. VII. Soorkrankheit bei Gänsen. Zeitschr. f. Infektionskr. der Haustiere 22:204.

Skinner, C. E.: 1947. The yeast-like fungi: Candida and Brettanomytes. Bact. Rev. 11:227.

Stovall, W. D.: 1939. Classification and pathogenicity of species of Monilia. Third Internat. Cong. for Microbiol., New York, P. 202.

Underwood, P. G.: 1955. Detection of crop mycosis (moniliasis) in chickens and turkey poults with a panendoscope. Jour. Am. Vet. Med. Assn. 127:229.

——, Collins, J. H., Durbin, G. G., Hodges, F. A., and Zimmermann, H. E., Jr.: 1956. Critical tests with copper sulfate for experimental moniliasis (crop mycosis) of chickens and turkeys. Poultry Sci. 35:599.

Worley, G., and Stovall, W. D.: 1937. A study of milk coagulation by Monilia species. Jour. Infect. Dis. 61:134.

Zürn, F. A.: 1882. "Durch Schimmelpilze hervorgerufene Krankheiten der Geflügels." Die Krankheiten des Hausgeflügels, Weimar, p. 129.

Sarcosporidiosis

Sarcosporidiosis appears to be neither widespread nor economically important in birds, at least in the United States. The striated muscles of mammals are involved principally, although reptiles and birds are sometimes affected. Among mammals, sarcosporidiosis is most likely to be found in sheep, swine, cattle, and horses. A few cases in man have been reported.

Occurrence. Erickson (1910) listed 8 orders, 13 families, 19 genera, and 20 species of birds as being affected. Some of the better known hosts are the chicken, domestic mallard, wild mallard, black duck, gadwall, American pintail, blue winged teal, shoveller, turkey vulture, and English sparrow. Ducks are especially likely to be affected, and Erickson states that all recorded cases are in puddle or dabbling varieties. Hall (1925), in listing

a case in a domesticated duck from a market in Washington, D.C., suggested that the infection might be of economic importance because the flesh would have been judged unfit for consumption, but there seem to be no other similar reports. Beaudette (1941) suggests that sarcosporidiosis is probably widespread but escapes notice because of the absence of symptoms in affected birds, and infection is not discovered unless the muscles are exposed. Reports of infection in chickens are uncommon and include Germany, Kuhn (1865); the United States, Stiles (1894) and Hawkins (1913); Hungary, von Ratz (1908); Bulgaria, Krause and Goranoll (1933); and Brazil, Reis and Nobrega (1936).

Symptoms, lesions, diagnosis. The lesions may be so small as to escape detection except by microscopic examination. When larger and present in considerable numbers, the musculature has a finely streaked or "wormy" appearance (Fig. 18.10). The individual sarcocysts, frequently called Miescher's tubes or sacs, are usually elongated masses, the long axes of which are parallel to the muscle fiber. Large cysts are sometimes referred to as Balbiania. The sarcocysts in the case reported by Stiles (1893) were 1.0 to 6.0 mm. in length by 0.18 mm. in breadth; in the case reported by Mathews (1930), they were 1 × 3 mm. These are larger than most cases reported in birds, but some reports in mammals range up to 5 cm. in length. An individual cyst when removed has a whitish or creamy appearance and is cylindrical with somewhat pointed ends and appears slightly lobulated on the surface. The cyst is divided by septa into compartments. The compartments in a mature cyst are filled with spores (Rainey's corpuscles) which are variously described as banana, crescent, sickle, or comma shaped. The spores are 3 to 15μ in length and 1 to 4μ in width. The compartments in the center of an old cyst tend to undergo degeneration. Mathews (1930) called attention to a variation in connective tissue and inflammatory response which was proportionately greater around cysts with more degeneration (Fig. 18.11). The sarcocysts start development within muscle fibers, but as they enlarge the fibers are destroyed and the larger cysts are intermuscular. Apparently the sarcocysts do not seriously injure their hosts. Most reports are on birds which were considered normal when killed. Very heavy infestations may possibly cause symptoms. Mice may be killed by heavy doses, and this suggests that the same may be true in larger animals and birds.

Etiology. Following the discovery by Miescher (1843) of Sarcosporidia in the muscle tissue of a mouse, they were first named Synchytrium miescherianum by Kühn (1865). This genus was, however, already in use to describe a group of funguslike organisms, and the genus

FIG. 18.10 — Severe sarcosporidiosis in a duck.
(Becker, Iowa State University.)

FIG. 18.11 — Sarcosporidia in breast muscle of chicken. ×150. (Biester, Iowa State University.)

Sarcocystis was established by Lankester in 1882. There are names of many species in the literature designated principally with respect to the animals infected, but the organisms have not always been host-specific, and the morphological differences are not substantial except in relation to size of the cysts. Alexeieff (1913) concluded that there was no reliable ground for distinguishing the supposed species of Sarcocystis and that all belong to one species, S. miescheriana. Hagan (1943) suggests that S. miescheriana has priority if all are considered as a single species, otherwise this term would apply only to infection in the pig. Sarcocystis rileyi, Stiles (1893), has been the term applied to the presumed infective protozoan in ducks since Riley told Stiles (1893) that the lesions noted by Walsh and Riley (1869) and believed by them to be similar to Cysticercus cellulosae of pork were identical with Stiles's Sarcocystis. Hawkins (1943) compared sarcosporidiosis in the chicken, the mallard, the domestic mallard, and the black duck and stated that all were apparently the same species.

The life history of Sarcosporidia is incompletely known and only brief mention is made here. Although Theobald Smith (1901, 1905) and others have reported transmission, particularly in mice, by feeding flesh containing mature sarcocysts, it is probable that this would not have been accomplished had the eating of feces been prevented. Spindler et al. (1946) established infection in swine by feeding feces and/or urine from animals and birds which had been fed muscles containing sarcocysts. Flesh from infected swine was fed to pigs, dogs, cats, rats, mice, and chickens. The feces and/or urine from these animals and birds were not infective for 15 days after consumption of the infected flesh but contained a stage of Sarcocystis thereafter which was infective for swine. These findings are in harmony with those of Negre from 1910 to 1918 (cited by Spindler et al., 1946). Scott's (1930, 1943) reports which include extensive surveys of the literature and Babudieri (1932) should be consulted by anyone involved in research on sarcosporidiosis. There is a latent period of at

FIG. 18.12 — Miescher's sacs, showing arrangement of "septa" (A, B, and C); their jointed structure (C, D, and F); and attachment of the Rainey's corpuscles (spores) to the "septa" (E and F). Sections were stained with Gram's stain. A, B, and C are from a naturally infected sheep. D, E, and F from a naturally infected wild duck; D, E, and F from a naturally infected wild duck. (Spindler, Proc. Helminth. Soc. Wash. 14:28, 1947.)

least six weeks between the time of exposure and the development of sarcocysts in the muscles. What happens during this time or the mode of escape of infective material from infected muscle is uncertain, but presumably those processes take place through the blood stream.

There has been considerable discussion as to whether the etiological agent is a protozoan or a fungus, and Wenyon (1926) suggested that the Sarcosporidia probably are fungi. Spindler and Zimmerman (1945) reported an investigation which showed that the infective agent in swine is a fungus and not a protozoan. An *Aspergillus* sp. was recovered by aseptically rupturing sarcocysts or Miescher's sacs into dextrose culture solution. Young pigs injected with or fed conidia harvested from the cultures harbored typical sarcocysts in the muscles at necropsy four to six months after exposure. A fungus like that injected

was recovered on cultures from the mature sarcocysts. Spindler (1947) prepared histological sections from a sheep and a duck in a study of the internal structure of Miescher's sacs. The sacs contain a network of jointed hyphaline structures (Fig. 18.12A, B, C, and D). The septa divide the sac into compartments (Fig. 18.12B and C). These structures appear jointed (Fig. 18.12C, D, and F). Rainey's corpuscles (spores) are shown attached to the septa (Fig. 18.12E and F). The staining reaction of the structures was found to be characteristic of fungi. This was confirmed by finding a delicate septate mycelium by heating Miescher's sacs from sheep, cattle, and birds in 30 per cent KOH solution and staining the residue with lacto-phenol cotton blue solution.

These findings in sarcosporidiosis will tend to redirect investigations and may hasten the procurement of definite information on many points which are not understood at present. Investigations are likely to be more intensive in mammals, particularly swine and sheep, than in birds because of the relative economic importance.

REFERENCES

Alexeieff, A.: 1913. Recherches sur les Sarcosporidies. 1. Étude morphologique. Arch. Zool. Exper., 51, 521. (Citation from Wenyon).

Babudieri, B.: 1932. I Sarcosporidi e le Sarcosporidiosi. Arch. f. Protistk. 76.421.

Beaudette, F. R.: 1941. Sarcosporidiosis in a black duck. Jour. Am. Vet. Med. Assn. 99.52.

Erickson, A. B.: 1940. Sarcocystis in birds. The Auk 57:514.

Hagan, W. A.: 1943. The Infectious Diseases of Domestic Animals. Comstock Publishing Co., Ithaca, N.Y. P. 489.

Hall, M. C.: 1925. Sarcocystis rileyi from the domesticated duck. Jour. Parasit. 11:217.

Hawkins, P. A.: 1945. Sarcocystis rileyi in the domestic fowl, Gallus gallus. Jour. Parasit. 29:500.

Krause, C., and Goranoff, S. A.: 1933. Ueber Sarkosporidiosis bei Huhn und Wildente. Zeitschr. Infektionskr. Parasit. Krank. und Hyg. Haustiere 45.261.

Kühn, J.: 1865. Untersuchungen uber die Trichinenkrankheit der Schweine. Inst. d. Univ. Halle 68. (Citation from Erickson).

Mathews, F. P.: 1930. Sarcosporidiosis in a duck. Jour. Am. Vet. Med. Assn. 76:705.

Miescher, F.: 1843. Ueber eigenthümliche Schläuche in den Muskeln einer Hausmaus. Ber. u. d. Verhandl. Naturf. Ges. Basel, V. 198. (Citation from Wenyon).

Reis, J., and Nobrega, P.: 1936. Tratado de doencas das aves. São Paulo, Brazil.

Scott, J. W.: 1930. The Sarcosporidia. A critical review. Jour. Parasit. 16:111.

——-: 1943. Life history of Sarcosporidia, with particular reference to Sarcocystis tenella. Wyo. Agr. Exper. Sta., Bul. 259:1.

Smith, T.: 1901. The production of sarcosporidiosis in the mouse by feeding infected muscular tissue. Jour. Exper. Med. 6:1.

——-: 1905. Further observations on the transmission of Sarcocystis muris by feeding. Jour. Med. Res. 13.429.

Spindler, L. A.: 1947. A note on the fungoid nature of certain internal structures of Miescher's sacs (Sarcocystis) from a naturally infected sheep and a naturally infected duck. Proc. Helminth. Soc. Wash. 14.28.

——-, and Zimmerman, H. E., Jr.: 1945. The biological status of Sarcocystis. Jour. Parasit. 31 (Dec. suppl.):13.

——-, ——-, and Jaquete, D S.: 1946. Transmission of Sarcocystis to swine. Proc. Helminth. Soc. Wash. 13:1.

Stiles, C. W.: 1893 Notes on parasites—18: On the presence of sarcosporidia in birds. Bur. Anim. Ind., U.S.D.A., Bul. 3:79.

——- 1891. Notes sur les parasites. Bull Soc. Zool. France. 19:160.

von Ratz, t.: 1908. Az izmokban éloskodo véglényekrol es a Magyar fauna ban elofordulo fajaikrod. Allattani kódeméneck 7:177. (Citation from Erickson)

Walsh, B. D., and Riley, C. V.: 1869. A measly wild duck. Am. Entomol. 1.89.

Wenyon, C. M.: 1926. Protozoology. Ballière, Tindall, and Cox, London.

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19

The Avian Leukosis Complex

Leukosis signifies a group of diseases which is characterized by autonomous proliferation of the precursors of blood cells. The term has largely replaced the older name leukemia (white bloodedness) principally because changes in the circulating blood, as implied by the name, are not an invariable pathologic feature.

Avian leukosis was first studied in a systematic way by Ellermann and fowl paralysis by Marek, at the beginning of the present century. Since both of these conditions often occur in the same flock, share a tendency toward tumor formation, and are of major economic importance, they are discussed under one chapter, without implying etiologic unity.

Although there is no universal agreement on the diseases which should be included in this chapter, the term avian leukosis is retained for its usefulness in focusing attention on the im-

portance of the problem to the poultry industry. The most interesting advances, namely the production of resistance-inducing-factor-free flocks and the ready transmissibility of the fowl paralysis syndrome, are of such recent date that their impact on final classifications can not be appraised at this time. Eventually this chapter must deal with the entire problem of avian viral tumors.

Most of the studies have dealt with the common fowl. The knowledge on corresponding diseases is incomplete in other species of birds, some of which have proved susceptible to experimental transmission of chicken leukosis. Reciprocal transmission of fowl paralysis from chicken to pheasant (and perhaps turkey) seems possible.

The development of our knowledge on the avian leukosis complex is outlined in the historical part; the various pathologic manifestations are discussed as independent entities as they present themselves

* Deceased April 16, 1965.

[512]

in practice, while cause and control are taken up from a common point of view.

HISTORY

The varied opinions on, and the practical importance of, the avian leukosis complex are reflected in the large number of references to this disease group in the literature. Comprehensive reviews have been prepared by Biely and Palmer (1932), Jármai (1934), Olson (1940), Engelbreth-Holm (1942), Furth (1946), Oberling and Cuérin (1954), Chubb and Gordon (1957), Biggs (1963), and Beard (1963a). The literature is selected from the standpoint of tracing the contributions which form the framework of our present concept of the avian leukosis complex.

Fowl paralysis. Under the term polyneuritis, Marek (1907) described a disease of chickens which was characterized by lameness and variable enlargement, due to mononuclear infiltration, of the peripheral nerves. In studying a similar disease in the North Atlantic States, Kaupp (1921) observed its frequent association with blindness. The first positive transmission experiments were reported by Van der Walle and Winkler-Junius in Holland (1924). The disease was studied from a pathologic point of view by Doyle (1926, 1928) and by Pappenheimer and his associates (1926, 1929a, b), who introduced the term neurolymphomatosis gallinarum. The last-mentioned authors pointed out the frequent association of visceral lymphomata originating from the ovary with infiltrative lesions in peripheral nerves, brain, and iris, and produced evidence of the transmissibility of the disease in about 25 per cent of the experimental birds. They believed neurolymphomatosis to bear no relationship to Ellermann's form of leukosis. The transmissibility of neurolymphomatosis has been questioned frequently in the literature (Olson, 1937).

Recently, however, Sevoian and his associates (1962, 1963, 1964) reported ready reproduction of the disease by inoculating day-old chicks of the Cornell S-line with field lymphomatosis tumors either as cellular or cell-free material, via the peritoneal or aerosol route. Vindel (1962) and Biggs and Payne (1963) likewise reported positive results in transmission experiments with Marck's disease. In a subsequent communication Vindel (1964) expressed the opinion that internal parasitism, especially coccidiosis, is epizootiologically related to Marek's disease, and that the evolution of the lesions in this disease may be brought about by a process of autoimmunization.

In a differential study of neurolymphomatosis and the lymphatic form of leukosis, Furth (1935) pointed out that the former is of frequent occurrence, associated with clinical paresis, and characterized by small-cell lymphocytic infiltration of the peripheral nerves and viscera, without blood or bone marrow involvement; the condition was found by him to be transmissible only by viable cells. Campbell (1954, 1956, 1961) and Darcel (1957) considered the condition to be purely inflammatory in character. Biggs (1961) agreed with this concept but was unable to differentiate histologically between "inflammatory" and "neoplastic" lymphoid reactions.

Fowl leukosis. Leukosis in the common fowl was first recorded by Caparini in 1896, according to Olson (1940). Extensive experimental studies of the condition were undertaken by Ellermann and his associates (1908, 1922, 1923), who recognized three general forms of avian leukosis, namely leukemic or aleukemic myeloid leukosis, intravascular "lymphoid," i.e., erythroid leukosis, and extravascular lymphatic leukosis, all of which were considered to be transmissible and caused by the same filterable virus.

This subject presented an intriguing problem to modern leukemia research in man and animals and was reopened in this country by Furth (1931a), who discovered a new strain of readily transmissible leukosis which conformed to Ellermann's intravascular "lymphoid" form; a rare subvariety with little blood involvement was described as anemic erythroleukosis (1931b).

Erythroblastosis. The characterization of

erythroleukosis was soon confirmed and extended, especially in Denmark, by Engelbreth-Holm and Rothe Meyer (1932) who suggested the term erythroblastosis and by Oberling and Guérin (1934) in France. Although "pure" strains of erythroid and myeloid leukosis were observed by Jármai (1930) and Nyfeldt (1934), the two forms tended to occur in a "mixed" form (Furth, 1931a) and were believed to be caused by the same filterable virus. In more recent times the extensive studies by J. W. Beard and his group on erythromyeloblastic leukosis (Eckert et al., 1951) resulted in the recognition of erythroblastosis as a morphologic and etiologic entity (Eckert et al., 1956).

Myeloblastosis (granuloblastosis). Leukemic myeloid leukosis of Ellermann was observed by Furth (1931a) and others in serial transmission experiments with strains of erythroblastosis. Olson (1936) discussed this disease under the heading of granuloblastic leukosis. The condition designated by Eckert et al. (1953) as erythromyeloblastic leukosis was identified morphologically as myeloblastosis by Burmester (in Eckert et al., 1953) and subsequently established as an etiologic entity by Beard (1956). While Brion and Fontaine (1963) pointed up the differences in physical, chemical, and antigenic properties between the viruses of myeloblastosis (strain A) and erythroblastosis (strain R), by electron microscopic examination these virus particles appeared to be of the same morphology (Bonar et al., 1963).

Lymphomatosis. Lymphatic leukosis represented the only extravascular form in Ellermann's classification of transmissible avian leukosis. Later his erstwhile collaborators, Andersen and Bang (1928), expressed doubt as to its transmissibility. That this disease constituted an independent nontransmissible entity was maintained by Mathews and Walkey (1929), who suggested the designation lymphadenoma and separated it sharply from neurolymphomatosis. This view was upheld by Feldman (1932), and by Feldman and Olson (1933), who used the term lymphocytoma for the aleukemic neoplastic

disease for which transmissibility had not been demonstrated and for which the type cell was the undifferentiated lymphocyte. Oberling and Guérin (1934) recognized differences between the nontransmissible extravascular forms and the transmissible intravascular ones. Furth (1935) agreed with the definition of lymphocytoma insofar as the "spontaneous" extraordinary enlargements of the liver were concerned, which condition he termed hepatolymphomatosis.

While it would appear from the foregoing statements that three separate entities have to be recognized, namely, neurolymphomatosis, transmissible erythromyeloblastosis, and nontransmissible lymphocytoma, other studies tended to break down the boundaries. Johnson (1932) was unable to differentiate lymphocytoma from the visceral lymphomata which had been described by Pappenheimer et al. (1926) in cases of neurolymphomatosis, and believed this association to be so common that he proposed the generic term lymphomatosis for the specific "neural" and "visceral" subdivisions. Furth (1933) developed a transmissible agent (strain 2) which was capable of causing what he also termed lymphomatosis and at times myelocytomatosis and endothelioma. To distinguish from neurolymphomatosis, he (1935) defined lymphomatosis as a rare disease which was not associated with clinical paresis; pathologically it was characterized by anemia, large-cell lymphocytic leukemia, and tumorous infiltrations of the same cell type in the visceral organs and occasionally the peripheral nerves; the disease proved easily transmissible by cell-free material. The term lymphomatosis was thus used in an etiologically and pathologically equivocal sense by Johnson and Furth. Experimental studies on the transmissibility of visceral lymphomatosis by Davis and Doyle (1947, 1949) and Davis et al., (1950) indicated that the incidence of the visceral type alone could be increased by inoculation. Supported by the analysis of extensive field material, this group of authors (Davis et al., 1947) considered

visceral lymphomatosis as a separate entity, distinct from neural lymphomatosis, a view held by the workers of the United States Bureau of Animal Industry Regional Poultry Research Laboratory (1951).

As stated, endotheliomata were observed by Furth (1933) in passages of strain 2. Starting with material from a case of neurolymphomatosis, Jungherr (1937) likewise observed endotheliomata. It would appear that this condition is sometimes an expression of lymphomatosis.

An uncommon hypertrophic osteopathy of chickens has been described in general ornithopathology under various terms such as hyperplastic osteitis (Reinhardt, 1930) and diffuse osteoperiostitis (Pugh, 1927). Transmission studies on this condition by Jungherr (1935) and Jungherr and Landauer (1938) tended to show that certain strains of lymphomatosis carry hypertrophic-osteopathic potentialities, for which the term osteopetrosis gallinarum was suggested.

Newer transmission experiments in a relatively pure form by Holmes (1958, 1959) and Fritzsche (1963) and pathologic and biochemical follow-up studies by Bell and Campbell (1961) suggested a specific virus as the etiologic factor (Holmes, 1961). On the other hand, the multiple cell response to a well-studied tumor virus, BAI strain A of myeloblastosis, included osteopetrosis (Heine et al., 1963), and field strains of visceral lymphomatosis likewise produced this condition in a good percentage of the inoculated inbred chicks (Burmester and Fredrickson, 1961).

Olson (1911) described a transplantable lymphoid tumor of the chicken with an unusually short incubation period that, according to gross and histopathologic features, would fall under the classification of lymphomatosis. Designating such a tumor as transplantable lymphosarcoma, Pentimalli (1911) made similar observations.

Intensive studies of the Olson tumor and similar highly malignant tumor strains by Burmester and his associates hold promise of clarifying the etiologic relationship of the various forms within the avian leukosis complex. In their hands (Burmester et al., 1944) the Olson tumor maintained its virulence almost unabated when frozen slowly and stored at $-65°$ to $-76°$ C for 391 days.

By intraperitoneal injections of young chickens with tumor tissues which morphologically were indistinguishable from lymphocytoma, visceral lymphomatosis, or "lymphoid tumors," Burmester and Prickett (1945) were able to develop several additional highly malignant tumor strains. Chickens immunized against a transplantable tumor were no more resistant to spontaneous neural or visceral lymphomatosis than comparable controls in the experience of Burmester (1947b) and Olson (1947). The latter author also found chickens spontaneously affected with neural lymphomatosis to be fully susceptible to implants of a lymphoid tumor.

Darcel (1953) compared the behavior of the Olson tumor (RPL-12) with two other strains isolated by Burmester and found them essentially similar. Intramuscular transplantation caused the appearance of intravascular neoplastic cells in the liver, but the mechanism of internal spread could not be established.

Transplantable tumor tissues and the plasma of lymphoid tumor-bearing chickens were shown to contain a filterable agent, capable of inducing visceral lymphomatosis and osteopetrosis after an incubation period from two to six months (Burmester et al., 1946a; Burmester, 1947a). The existence of filterable agents producing lymphoid tumors and osteopetrosis was confirmed by serial passage in chickens (Burmester and Cottral, 1947). Although finding variations in the transmissibility of the disease from different donors (Burmester and Dennington, 1917), avian lymphomatosis could be propagated with cellular as well as cell-free preparations (Burmester, 1947b). In this series the incidence of osteopetrosis and neural lymphomatosis was surprisingly low, which suggested different etiologic agents for the latter conditions. Burmester (1947c) ex-

pressed the opinion that many cases of natural lymphomatosis carry masked *lymphoid tumor agents*.

In quantitative follow-up studies, Burmester and Gentry (1956) made the interesting observation that the virus of visceral lymphomatosis, as represented by plasma filtrates of RPL-12, tended to cause early intravascular erythroblastosis, when given in high doses, and late extravascular lymphomatosis when given in low doses. These changes were accompanied by a low, but significant, incidence of osteopetrosis in all inoculated birds, especially the males. This dual cytotropism maintained itself in pathogenicity tests.

In detailed studies of the pathogenicity of RPL-12, Gross et al. (1959) showed that the virus may induce not only visceral lymphomatosis but also erythroblastosis, hemangiomatosis, and osteopetrosis. From the same data Burmester et al. (1959a, 1960a) brought out the importance of the host-virus interrelationship on the pathologic response, especially with respect to age and genetic background of the host, and source, dose, and route of inoculation of the virus. For the principal neoplastic manifestation, i.e., erythroblastosis and visceral lymphomatosis, age of host and virus concentration in inoculum proved statistically to be the important variables affecting the responses (Gross et al., 1962). Partial fixation of pathologic response could be obtained by selection of donors (Walter et al., 1963). In the hands of Burmester et al. (1959b), even so-called "pure" strains of erythroblastosis (R) and and myeloblastosis (BAI-A), produced all combinations of the oncogenic spectrum, inclusive of "renal carcinoma," with the exception of myeloblastosis and visceral lymphomatosis in the same bird. Both diseases were found to be transmitted also by contact, a property which was formerly attributed only to lymphomatosis.

To test the relationship of RPL-12 to field virus tumors, Burmester and Fredrickson (1964) collected material from 22 widely separated flocks in the U.S. and induced better than 50 per cent neoplasm

mortality in L 15 1 inoculated chickens from 9 sources. According to Burmester (1962) these data pointed to a single causative virus but with minor continuous differences for erythroblastosis, visceral lymphomatosis, osteopetrosis, fibrosarcoma, hemangioendothelioma, and nephroma. Myeloblastosis, myelocytomatosis, and neural and ocular lymphomatosis were not observed, in spite of the fact that some of the donors were so affected.

The latter point as to neural and ocular lymphomatosis was of particular interest in light of the reports by Sevoian and his associates on the experimental reproduction of the neural and visceral form of lymphomatosis in Cornell S-line chicks (Sevoian and Chamberlain, 1962, Sevoian et al., 1962), and the preferential occurrence of these forms in young and in old chickens, respectively, as seen in the field (Sevoian and Chamberlain, 1963). In their opinion (Sevoian et al., 1963a) these points furnished new support for the etiologic unity of avian lymphomatosis.

In contrast to the above neoplastic concept of lymphomatosis, Campbell (1954, 1956), Darcel (1957), and Chubb and Gordon (1957) interpreted the term lymphomatosis as representing a chronic inflammatory condition, with neural, ocular, or visceral localizations. Neoplastic aberrations of the lymphoid series were designated as lymphoid leukosis. Histogenetic considerations formed the primary basis of differentiation. To avoid confusion between inflammatory lymphomatosis and neoplastic lymphoid leukosis, the former designation was to be dropped and replaced by Marek's disease (Biggs, 1961). Unfortunately this term is little understood in this country.

Myelocytomatosis. Aleukemic myeloid leukosis was considered by Ellermann (1923) as a subvariety of transmissible myeloid leukosis. The disease was ordinarily associated with tumor formation. Pentimalli (1915) apparently first described a spontaneous chicken tumor which was composed almost exclusively of myelocytes with the characteristic granu-

lations of the mature eosinophil and heterophil. Mathews (1929), believing the tumor to be analogous to chloroma in man, suggested the term leukochloroma. He failed to show transmissibility of the condition. Feldman (1932) regarded it as a definite neoplastic process which he classified as myelocytoma. In a passage experiment with lymphomatosis strain 2, Furth (1933) observed cases of myelocytoma with myelocytic blood involvement (myelomatosis). This apparently constitutes one of the few recorded instances of transmissible myelocytomatosis.

Nephroblastoma. This tumor was formerly known as embryonal nephroma, resembling the Wilm's tumor in man and was considered to represent a developmental aberration. Recently it has been produced with certain strains of leukosis virus.

Carr (1956) first reported the induction of renal adenocarcinoma with Engelbreth-Holm's ES1 strain of erythroblastosis. Only chicks inoculated intramuscularly under 2 weeks of age manifested this lesion about 4 weeks later. Survivors over 3 weeks old (Carr, 1959) had occasionally true, transplantable metastases. Among many other tumor viruses tried, only the MH2 Murray and Begg reticuloendothelioma strain had a similar oncogenic attribute. The author pointed out that this was the second case of a mesenchymal virus attacking immature kidney tissue which is of mesodermal origin.

In long range experiments with established erythroblastosis (R) and myeloblastosis (BAI-A) strain, Burmester et al. (1959a) observed the latter to produce not only myeloblastosis, but also visceral lymphomatosis, renal carcinoma, and osteopetrosis. Subsequently, Walter et al. (1962) studied the transmission and pathology of this virus-induced tumor. He proposed the term nephroblastoma as a more accurate designation to indicate that primitive nephrogenic tissue is capable of differentiating into both epithelial and connective tissue. By transplantation it was possible to alter the oncogenic potenti-

alities of the BAI-A strain to an almost "pure" nephroblastoma strain. Pathologically, nephroblastoma is essentially composed of malformed nephrons with occasional admixtures of cartilaginous and osteoid tissue. In trying to explain this biologic response de Thé et al. (1962) assume that the myeloblastosis virus acts in the differentiation of nephrogenic cells like a normal inductor, but with abnormal results. The detailed morphologic studies of Ishiguro et al. (1962) have shown that the growth originates in nephrogenic elements which are residual in the postembryonic kidney of the fowl.

The unitarian view. In general the extended investigations of the avian leukoses by Furth tended to show that each transmissible strain represents an etiologic unit because of its more or less definite, if occasionally wide [e.g., strain 2 (Furth, 1933)], pathologic range. Quite in contrast to this concept is the unitarian point of view which assumes that a single etiologic agent is responsible for all of the various pathologic manifestations of the avian leukosis complex. On the basis of transmission experiments with neurolymphomatosis and fowl leukosis, Patterson and co-workers (1932, 1931, 1936) concluded that "fowl leukosis" can be subdivided into erythroid, myeloid, lymphoid (including lymphocytoma), nerve, eye, and mixed types, all of which were considered to be different expressions of the same transmissible disease.

Johnson (1931), in continuation of his work on lymphomatosis (1932), considered erythro- and myeloleukosis likewise to be due to the causal agent of lymphomatosis, and suggested the inclusive term hemocytoblastosis. This term was based upon the concept of Jordan and Johnson (1935) and Jordan (1936) that the hemocytoblast of the marrow stroma is the primitive reticulum stem cell which gives rise to both the erythroblastic and granuloblastic series. This was in conformity with Ringoen (1931), and in contrast to the theory of attributing erythrogenesis to the endothelial cells of the venous sinuses, advanced

by Doan et al. (1925). Hall and associates (1941, 1943) developed a strain of hemocytoblastosis from original neurolymphomatosis material which, in the hands of the U.S. Regional Laboratory workers, showed the characteristics of myeloblastosis (RPL-1).

Emmel (1939) also advanced a unitarian view and likewise used the inclusive term hemocytoblastosis for an increase or decrease in leukocytes accompanied by the appearance of immature and degenerative blood cells in the circulating blood. Blount (1939) has shown that similar blood pictures occur in physiologic transition stages from embryonic to adult life and in various unrelated diseases.

Relation of leukosis to virus tumors. Leukosis was thought to be unrelated to transmissible sarcomas and similar tumors of the fowl which have been studied extensively by Rous, his associates, and other workers (for ref. see Claude and Murphy, 1933; Foulds, 1934). Oberling and Guérin (1933a, b), however, presented evidence on the production of malignant tumors of the Rous type with the virus of transmissible leukosis; a similar polyvalent strain was studied by Rothe-Meyer and Engelbreth-Holm (1933) and by Engelbreth-Holm and Rothe-Meyer (1935). A strain of leukosis described by Jármai (1935) produced fibrosarcomas at the point of injection in leukosis-refractory birds.

These observations stimulated a large amount of investigational work on the relation of leukosis to sarcoma, the results of which seemed to indicate that simple, mixed, and complex strains occur (Furth, 1936a).

Simple or pure strains maintain their pathologic identity in successive passages, as exemplified by the Rous sarcoma or erythroleukosis (strain 1) of Furth (1931a), which Stubbs (1938) tested for tumor-producing properties over several years, with negative results. A recently isolated Canadian strain of erythroleukosis likewise failed to produce neoplasm at the site of inoculation (Wickware, 1943, 1946).

If leukosis and sarcomalike processes occur in the same donor and prove to be dissociable in subpassages, the conception of "mixed" strain is applicable. Furth (1936b) observed an osteochondrosarcoma (strain 12) in a bird inoculated with lymphomatosis (strain 2) (Furth, 1933) and showed that in successive transplantations both pathologic components occurred either alone or in combination. However, later culture studies of the virus in vitro by Furth and Breedis (1935) suggested that it may have been a complex strain. From a spontaneous ovarian tumor which had both lymphomatous and sarcomatous characters, Jungherr (1937) developed agents of lymphomatosis and sarcomatosis, the latter of which was carried as such through many subpassages by Cole (1941).

Complex strains apparently are due to a single agent which can stimulate both primitive blood cells and fibroblastic cells. Stubbs and Furth (1935) described the interesting strain 13 which produced sarcoma on subcutaneous or intramuscular inoculation, and diffuse endothelial sarcomatosis in the blood-forming organs associated with erythroleukosis, when injected intravenously. The strain studied by Oberling and Guérin (1933a, b), Rothe-Meyer and Engelbreth-Holm (1933), and Jármai (1935) may have been of a similar order (Furth, 1936a).

A major impetus has come from the observations of Duran-Reynals (1940, 1941, 1947) that the classical Rous sarcoma virus is capable of assuming hemorrhagic, neurotropic, and osteopetrotic properties, after experimental passage through newly hatched ducks and chickens. These findings suggested a necrotizing effect of the virus on the vascular endothelium, but Carr (1962) believed that the "hemorrhagic disease" induced by Rous virus 1 and other avian tumor viruses, tested by him, was primarily associated with pre-existing foci of extramedullary hematopoiesis and not in itself evidence of a viral necrotizing property. In studying the genetic resistance of fowls to Rous sarcoma virus, Greenwood and Carr (1951) obtained increased resistance to artificial

sarcoma infection only at the expense of an increased death rate from fowl paralysis, and suggested combined consideration of the sarcoma-leukemia-fowl paralysis viruses.

In a comprehensive review of the role of viruses in the production of cancer, Oberling and Guérin (1954) pointed out that even malignant tumors which are clearly related, such as the Rous and the Fujinami sarcomas, are distinguishable, since morphogenesis may be a consequence of the narrow cytotropism of the viruses involved. The authors had no doubt as to the basic leukemic nature of the various forms of avian lymphomatosis and believed many closely related viruses to be involved. Beard (1957a) has summarized the evidence for the relationship of avian leukosis to avian sarcomas, particularly on the basis of cross virus neutralization tests. He believes that preliminary studies warrant the interpretation of an etiologic interrelationship between various forms of the leukosis complex and certain avian tumors. Clarification of the etiology can come only from the application of modern virologic techniques which are available, but "failure of cell-free transmission cannot be accepted as evidence that a given tumor is not of viral etiology" (Beard, 1957b).

Research on Rous sarcoma virus has recently entered entirely new territories. Following the original demonstration of the pathogenicity of Rous virus for albino rats by Svet-Moldavsky (1957; with Skorikova, 1960) and Zilber and Kryukova (1957), Ahlström et al. (1963) reported the transmissibility of the Schmidt-Ruppin strain to young rats, mice, guinea pigs, and rabbits, whereas the Mill Hill strain did not induce such tumors, although these strains showed serologic relationship by cross protection tests. Takes in young rhesus monkeys were first obtained by Munroe and Windle (1963). By intravenous inoculation of a "standard" Rous virus strain, Burmester and Walter (1961) were able to induce not only the expected disease, but also visceral lymphomatosis and erythroblastosis. With Fontes, they (Burmester

et al., 1960b) even obtained evidence of Rous sarcoma transmission by contact, an observation not previously recorded. However, the authors did not consider this as critical evidence for a single multipotent virus because of a possible mixture of viruses in the starting inoculum. This possibility was recently confirmed by Rubin and Vogt (1962) who found the high-titer Bryan strain of Rous sarcoma virus to contain also the Rous associated virus which has the physical and biologic attributes of the lymphomatosis-myeloblastosis agents.

In a recent survey of avian virus growths and their etiologic agents, Beard (1963a) emphasizes that these tumors are all of mesodermal origin and are characterized more by the growth potential of the cell involved than by the virus. Avian tumor viruses are genetically unstable: their responses are affected by experimental adaptation and spontaneous mutation. With particular reference to the avian leukosis complex, he (1963b) points out that transmission of leukosis material results in a large variety of conditions, but that similarities in biologic characters of solid and hematopoietic tissue tumors reveal their etiologic interrelationship. He takes issue with the details of data collected over the past half century which have created the illusion of complexity, and questions whether any strain can ever be designated as "the virus" of lymphomatosis because the latter is essentially a pathologic entity.

Nomenclature. The boundaries of the disease group are indistinct. In the restricted sense, *the avian leukosis complex includes primarily those diseases which are characterized by autonomous proliferation of essential blood-forming cells.* This definition may have to be extended to include viral tumors derived from the mesodermal layer of the embryo, including the hematopoietic, connective, bone-forming, endothelial, and mesothelial tissues and certain epithelial cells of kidney and ovary (Beard, 1963b). The common features for this disease group, according to Beard (1957a), are that each form represents a

pathologic entity; combinations of these forms may occur in nature in the same flock or bird; various mixtures can be transmitted by cellular or filtered material; and, most inexplicably, certain forms may be obtained from source materials which do not show the original pathologic features.

A tentative pathologic classification, without implying etiologic unity, was suggested by a committee (Jungherr et al., 1941) cooperating with the United States Regional Poultry Research Laboratory, East Lansing, Michigan. The various forms of the disease complex, which were understood to occur in leukemic, subleukemic, or aleukemic variety, were grouped into lymphomatosis, with either neural, ocular, visceral, or osteopetrotic localization; erythroblastosis; granuloblastosis; myelocytomatosis; and sarcomatosis.

Beard (1957a) conceived the avian leukosis complex as a group of diseases, with lymphomatosis and its neural, ocular, and visceral localizations on the one hand, leukemias and sarcomas on the other, and osteopetrosis as a potential link between the extremes of the pathologic spectrum (Fig. 19.1).

Chubb and Gordon (1957) suggested a modified conception, based upon the histogenetic interpretation of Campbell (1954). Leukosis was divided into erythroleukosis; myeloid leukosis of diffuse or discrete distribution, the latter identical with myelocytoma; and lymphoid leukosis again of diffuse or discrete distribution,

FIG. 19.1 — The major conditions of the avian leukosis complex. The connecting curves indicate, in part, the diversity of combinations occurring naturally, or transmissible by experimental means. (Beard, 1957a, Ann. N.Y. Acad. Sci.)

the latter identical with lymphocytoma. Lymphomatosis was divided into visceral, neural, and ocular forms. Osteopetrosis was placed into a separate category. The term lymphomatosis in the above sense has since been replaced by Marek's disease (Gordon, 1960; Campbell, 1961; Biggs, 1963). However, the distinction between inflammatory and neoplastic forms is not tenable because of the limitations of the histopathologic approach. Although much has been written about classification, the greatest need is for large scale critical experiments to show whether control measures against known agents such as RIF will alter the susceptibility of a flock to agents which may be implicated in Marek's disease.

A simplified modification (with scientific and common equivalents) of the 1941 proposal has been suggested by Cottral (1952) and is used here. It comprises, neural, ocular, and visceral lymphomatosis, osteopetrosis, erythroblastosis, myeloblastosis, and myelocytomatosis. To this has to be added nephroblastoma. It has the advantage of designating the individual pathologic forms, and thereby avoiding any implication as to etiologic unity.

In rendering a diagnosis emphasis should be placed upon stating the specific form or forms found and preferably also the method of arriving at such diagnosis, rather than generic or group designations such as lymphomatosis or avian leukosis complex.

NEURAL LYMPHOMATOSIS

Synonyms. Fowl paralysis, range paralysis, polyneuritis (Marek, 1907), neuritis (Doyle, 1926), neurolymphomatosis gallinarum (Pappenheimer et al., 1926), neurogranulomatosis (Lerche and Fritzsche, 1934), Marek's disease (Biggs, 1961).

Paretic symptoms may be observed as accompanying a variety of diseases such as tuberculosis, staphylococcosis, fowl cholera, helminthiasis, coccidiosis, botulism, avitaminosis, lead and salt poisoning, etc. To differentiate such conditions from true fowl paralysis, as discussed below, the for-
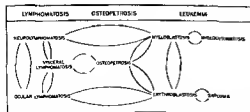
FIG. 19.2 — Neural lymphomatosis. Typical clinical position in advanced case. (L. P. Doyle, Jour. A.V.M.A.)

mer may be grouped under the term symptomatic paralysis, according to Bayon (1932).

Occurrence. The disease has been observed in every major poultry-producing country of the world. It attacks primarily young birds between 2 and 5 months of age, but has been seen as early as 3 weeks of age and as late as the second year of production. All breeds and both sexes are susceptible. The losses are variously estimated from 5 to 25 per cent in affected flocks. Under practical conditions the disease often makes its appearance when the birds are first turned out on range; then, after a period of quiescence, the disease apparently resumes its course in the visceral form when the birds are put into the laying houses.

Symptomatology. The clinical signs of the disease are usually those of asymmetric progressive paresis of the leg, wing, or neck, the paresis being either spastic or flaccid in character. In the beginning the affected leg may show inward curving of the toes, weakness, or incoordination. Later on, the bird has a tendency to hold one foot stretched forward or backward, a position which is quite characteristic (Fig. 19.2). If both legs are affected the animal moves with difficulty, in a squatting position. Involvement of a wing is indicated by drooping of the extremity (Fig. 19.3); if one tries to spread the wings the dis-

FIG. 19.3 — Involvement of a wing, as indicated by drooping of extremity. (L. P. Doyle, Jour. A.V.M.A.)
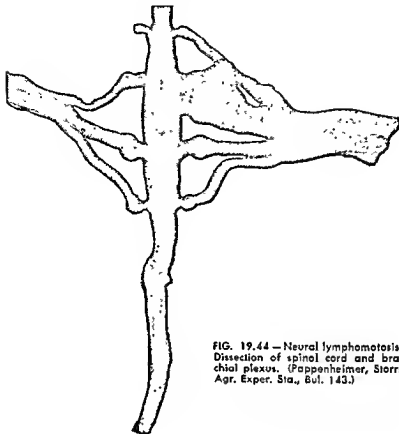
FIG. 19.44 — Neural lymphomatosis. Dissection of spinal cord and brachial plexus. (Pappenheimer, Storrs Agr. Exper. Sta., Bul. 143.)

eased one often gives the impression of increased resistance. Paresis of the neck may be suggested by low carriage of the head and incipient torticollis; affection of the deep muscles and the nerves, especially the vagus, may lead to dilatation of the crop and gasping symptoms. Durant and McDougle (1945) considered soiled, damp, "front" feathers under the beak and along the throat to be a sign of neural lymphomatosis and attributed it to involvement of the posterior cranial and the anterior cervical nerves. Locomotory disturbances are often associated with systemic reactions such as loss of weight, paleness, anorexia, and diarrhea, although the appetite not infrequently remains good. The signs of true fowl paralysis are not specific and vary widely in intensity.

Pathology. The gross anatomic features of the disease are characterized by localized or occasionally diffuse grayish, soft swellings of the peripheral nerve trunks. The femoral portion of the sciatic trunk is commonly affected (Fig. 19.4A). This nerve can be observed easily by lifting up the large triangular adductor muscles on the median surface of the thigh under which its two strands run parallel with the femoral artery. The normal nerve is uniform in width, white, and cross-striated. Loss of striation is suggestive of the disease. Bilateral comparison permits the detection of mild alterations. For detailed examination the distal and proximal ramifications of the nerve should be exposed. Changes may be seen in the intrapelvic part of the trunk, the so-called sciatic plexus, which originates from four spinal roots and is located under the middle lobe

FIG. 19.4B — Experimental neural lymphomatosis of brachial plexus. (Courtesy Dr. M. Sevoian, University of Massachusetts.)

of the kidney. The lumbar and celiac plexuses stand out very prominently, if affected. Some portion of the brachial plexus may be involved, although there is no constant correlation between neuromuscular disturbances and regional nerve lesions. Vagus affection is apt to occur if respiratory signs are present. The dorsal ganglia of the peripheral nerves, especially in the region of the brachial plexus roots, often undergo grayish enlargement which may extend into the spinal cord and form tumorlike masses (Fig. 19.5). It is of great interest that, after years of trials by other investigators, Sevoian et al. (1962) succeeded in reproducing such lesions by the intraperitoneal inoculation of day-old chicks from the Cornell S-line, with field lymphomatous tumors (Fig. 19.4B).

In uncomplicated cases of fowl paralysis the visceral organs appear normal. The spleen is usually of normal size; the thymus lobes may show glandular enlargement (Jungherr, 1933). According to Johnson (1934) the bone marrow of the ulna and radius, which ordinarily is fatty and aplastic in mature birds, may revert to a

state of hyperactivity often seen in young normal birds.

Systemic lesions were described by Blakemore (1939) and Blakemore and Dalling (1939), who, in transmission experiments of neurolymphomatosis with liver emulsions, observed whitish, ill-defined areas especially in the heart and liver substance corresponding microscopically to focal necrosis followed by infiltration with various mononuclear cells. Typical nerve lesions developed in protracted cases. Glover (1940) made similar observations in chicks experimentally inoculated with neurolymphomatosis from pheasants. Asplin (1944) made the interesting observation that a "chick disease" of known viral etiology was amenable to sulfonamide therapy. In further studies Asplin (1947a) failed to substantiate any etiologic connection with lymphomatosis (1947b) and may have been dealing with the Gal (Gallusadenolike) virus (Burmester et al., 1960d; Sharpless and Jungherr, 1961).

Campbell (1956) advanced the view that the initial change in the liver is focal, fibrinoid necrosis followed by re-
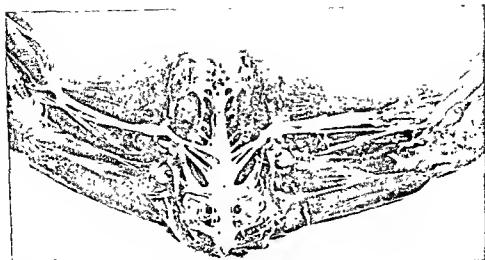
FIG. 19.5 — Neural lymphomatosis. Formol portion of right sciatic nerve thickened. (F. D. Patterson, Iowa State University.)

FIG. 19.6 — Neural lymphomatosis. Section of sciatic nerve. (Poppenheimer, Storrs Agr. Exper. Sta., Bul. 143.)

active lymphocytic infiltrates.

On microscopic examination affected nerves present either follicular or diffuse infiltration with mononuclear cells (Fig. 19.6). The majority of the pathologic elements are indistinguishable from lymphocytes in the circulating blood and correspond to so-called small round cells; others have the character of plasma cells, large mononuclears, or histocytes. The lesions may be associated with edema, myelin degeneration, and reactive increase of the Schwann sheath cells, but axonal degenerations are rare. Spinal and sympathetic ganglia undergo similar infiltrative changes. The disease also affects the central nervous system where it brings about either compact perivascular rings of small densely staining lymphoid cells or submiliary nodules composed of such cells and paler elements, probably of glial origin (Pappenheimer et al., 1926). Granulocytic reactions are not characteristic. The intensity of the central nervous system lesions seems to vary inversely with their development in the peripheral nerves. Ordinarily, involvement of the central nervous system is secondary to that of the peripheral, but one has to recognize the occurrence of a "central" variety of neural lymphomatosis not associated with peripheral nerve lesions. In a recent case of "torticollis," the auditory nerve presented the principal lesions. Siller (1960) described an unusual case of persistent cloacitis (vent gleet) which was characterized by megacolon, typical gross changes in the accompanying intestinal nerve, and microscopic changes in the central nervous system.

Microscopic lesions frequently occur in grossly normal-appearing nerves. Microscopic examination thus constitutes the most sensitive method of diagnosis available at the present time. Due to the focal distribution of the lesions, the accuracy of the method is proportional to the number of levels of the nervous system examined. In a detailed study of lymphoid tissue in some splanchnic nerves, Oakberg (1950) found all of the birds over 20 days

of age in his material to show some basic microscopic lesions and believed them to indicate specific infection.

The intensity of the lesions and their stage of development in the peripheral nerves are variable as brought out by Wight (1962a) who recognized three types, i.e., cellular infiltrations with mature lymphocytes, edema with less cellular infiltration, and lymphoblastic infiltration with mitoses. The second type was most common. In a companion study of the central nervous system, he (1962b) found perivascular cuffs also in the controls and cautiously considered such lesions as an adjunct to the pathologic picture of fowl paralysis. Lymphoblastic infiltrations were often associated with neoplastic disease. There was no evidence of primary neuronal nor myelin degeneration.

Electron microscopic study of the peripheral nerves by Deutsch and Siller (1961) largely confirmed the histologic observations but also disclosed an endoneural fibrosis which may be the pathologic substratum of the clinical signs.

Hematology. In a basic publication Pappenheimer et al. (1926) stated that, although the blood was not subjected to a systematic study, no indications of significant blood changes were observed. Absolute and relative lymphocytosis was noticed by Bayon (1931) in four acute cases. Johnson and Conner (1933) reported an absolute increase in leukocytes, a relative increase of monocytes and basophils, and the appearance of "budded" lymphocytes. Observing an early polymorphonuclear and late lymphocytic leukomorphonuclear and late lymphocytic leukocytosis, Seagar (1933) made this the basis of the so-called cyto-diagnosis of fowl paralysis. Blount (1934), Dobson (1934), Hamilton (1934), and Blakemore (1934) pronounced this test unreliable and nonspecific. Gibbs (1934) confirmed the observations of Seagar, but considered the blood changes of little diagnostic assistance, on account of normal hematologic irregularities. In his pathologic characterization of fowl paralysis, Furth (1935) pointed out the usual lack of blood and bone mar-

row involvement. Jungherr (1934) made serial hematologic examinations of experimentally injected and control birds; the mild evidence of leukocytosis and lymphocytosis found in affected birds was regarded to be statistically insignificant.

Differential diagnosis. Other morbid conditions summarized under the term symptomatic paralysis (Jungherr, 1942) may be accompanied by paretic signs. In questionable cases the specific diagnosis of neurolymphomatosis rests upon the microscopic demonstration of the pathognomonic lesions in the peripheral nerves Brooder chicks may show in the sciatic trunks, as in other organs, slight granulocytic mononuclear foci which are probably expressions of ectopic hematopoiesis and of no diagnostic significance. Neuromalacia, or ariboflavinosis, is characterized by bilateral diffuse graying swelling of the sciatic trunks, which on microscopic examination exhibit severe myelin degeneration and occasionally mild perivascular proliferation of the adventitial cells (Phillips and Engel, 1938). The microscopic lesions of central neurolymphomatosis and avian encephalomyelitis (Jones, 1934) are similar in character except that the latter have a special predilection for the gray matter in the brain (Jungherr, 1935). The "crooked-toes" condition described by Norris et al. (1940), apparently related to mechanically unsuitable floors, is not associated with nerve alterations. Intracranial gliomas may occasionally give rise to paralytic signs (Jungherr and Wolf, 1939). The principles involved in the neuropathologic differentiation of symptomatic paralysis in fowl have been summarized by Jungherr (1953).

Neural Lymphomatosis in Other Species

A disease pathologically indistinguishable from the corresponding condition in the common fowl has been observed in pheasants by Jungherr (1939) (Fig. 19.7). An unusual feature was the finding of localized areas of muscle degeneration, especially in the flexors of the leg, which led to macroscopic "tigering" of the affected muscle and microscopic Zenker's degeneration associated with regenerative phenomena (Fig. 19.8), similar to the muscle changes sometimes observed in young chickens by Potel (1938). Harriss (1939) and Johnson (1941) apparently transmitted fowl paralysis to pheasants. A similar affection of both neural and muscular tissues has been described in turkeys
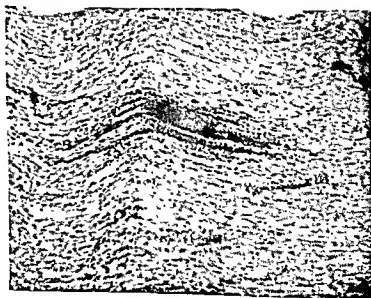
FIG. 19.7 — Neural lymphomatosis in pheasants. Section of sciatic nerve.

FIG. 19.8 — Neural lymphomatosis in pheasants. Section of leg muscle showing Zenker's degeneration.

by Andrewes and Glover (1939), who regarded it as representing true fowl paralysis in another species of bird. Glover (1940) transmitted turkey paralysis to chicks and believed it to be caused by the same agent which is responsible for chicken paralysis. Sevoian et al. (1963a) found two flocks of turkeys of which the poults were just as susceptible to the lymphomatosis virus as the S-line chicks. In turkeys the spontaneous disease must be differentiated from occasional cases of neurofibromatosis (Helmboldt and Frazier, 1962). Aside from chickens and turkeys, Wight (1963) quoted reports from the literature on neural lymphomatosis in pheasant, pigeon, duck, goose, canary, budgerigar, and swan. He himself observed the disease, associated with lymphoid leukosis, in Japanese quail (Coturnis coturnis japonica) which had been raised near infected fowl. Cottral and Winton (1953) found, in ducks, a condition simulating neural lymphomatosis.

OCULAR LYMPHOMATOSIS

Synonyms. Blindness, gray, glass, pearly-, fish-eye, iritis (Pappenheimer et al., 1926); epidemic blindness (Findlay and Wright, 1933); uveitis (Doyle, 1928); Marek's disease (Biggs, 1961).

As already noted, Kaupp (1921), Doyle (1926), and Pappenheimer et al. (1926) have observed the frequent association of blindness with cases of neural lymphomatosis. Findlay and Wright (1933) and Jaensch and Lerche (1933) believed epidemic blindness to be an expression of fowl paralysis. Similar epizootiologic observations were made by De Boer (1934a, b) in Holland, and Magnusson (1935) in Sweden. Although Upp and Tower (1936), on the basis of crossbreeding studies with paralyzed and blind birds, regarded blindness as an independent disease, later studies by McClary and Upp (1939) showed a high incidence of iritis in the progeny of iritis-affected parents. Bayon (1936) gave a pathologic description of a primary iridocyclitis in fowl said to be distinct from ocular lymphomatosis. Starting with affected iris material, Jungherr (1937) apparently developed transmissible strains of neuro-visceral lymphomatosis. Probably the most elaborate breeding experiments with birds, of which either one or both parents had ocular lymphomatosis, were carried out by Lee and Wilcke (1941), who found that the offspring showed a significantly higher incidence of various forms of the avian leukosis complex than that of nonaffected control

stock. Nelson and Thorp (1943) found pearl-gray irises with irregular pupillary borders to be extensively infiltrated with lymphocytes. They believed the early stages of the disease, without pupillary changes, to be represented by depigmentation and vascular congestion of the iris. Using chicks from iritic dams as virus donors, Durant and McDougle (1945) transmitted neural lymphomatosis by direct blood transfusion to susceptible chicks.

On the other hand, Ball (1944), working with Single Comb White Leghorns, found depigmentation of the iris, with round regular pupils, to be common and due to various factors such as lack of, or the presence of inhibitors of, carotenoid pigments in the diet, and high egg production A large proportion of such depigmented irises showed lymphocytic infiltration on microscopic study (Ball, 1945). Breeding from birds so affected failed to indicate a significant relationship between iris color of the dam and mortality from neoplastic or other diseases in her progeny (Ball and Cole, 1946). Nelson (1947) likewise believed that ocular lymphomatosis should be diagnosed by criteria other than depigmentation

The suggestion by Bullis et al. (1950) that ammonia fumes arising from deep litter in poorly ventilated brooder houses may cause eye lesions not readily distinguishable from ocular lymphomatosis, and their experimental production in the reviewer's laboratory by Ellis and Winn (1950) and by Faddoul and Ringrose (1950), throw doubt upon the occurrence

FIG. 19.9 — Ocular lymphomatosis. The eye on the left shows annular, that on the right, spotty, depigmentation of the iris.

of ocular lymphomatosis in young birds. Pathologically these so-called ammonia burns resemble the primary iridocyclitis described by Bayon (1936) but usually show the added feature of epithelial defects in the cornea. With the recent recognition of iritis and cataract as a possible sequence to avian encephalomyelitis (epidemic tremor) by Peckham (1957) and Bridges and Flowers (1958), this malady should be considered in differential diagnosis. Rigdon (1959), however, found also such cataracts in chickens with lymphomatosis.

Occurrence. Ocular lymphomatosis occurs primarily in flocks affected with the neural form but, on the whole, somewhat later during the life of the birds, primarily during early maturity. Accurate differential diagnosis is important. Variations in incidence are not considered as a dependable sign of the prevalence in the flock of other manifestations of the avian leukosis complex.

Symptomatology. Familiarity with the signs is necessary for those concerned with selection of breeding birds. Refinements in clinico-diagnostic methods, perhaps with the aid of an ophthalmoscope, should be helpful.

In the normal eye the iris has either a clear bay or orange color; the pupil is circular and has the power of ready accommodation to light intensity. The iris sometimes shows fine radial lines or clefts, which are probably in the nature of congenital defects

Ocular lymphomatosis manifests itself by concentric annular or spotty depigmentation or by diffuse bluish-gray fading of the iris in one or both eyes (Fig. 19.9). The pupil becomes irregular to the extent of showing angular indentations and gradual loss of light accommodation. In advanced stages the iris presents a diffuse grayish opacity with pin-point pupil (Fig. 19.10). The anterior chamber of the eye sometimes contains slightly turbid exudate which leads to increased convexity of the cornea as in glaucoma.

Pathology. The specific pathologic fea-

FIG. 19.10 — Ocular lymphomatosis. Uveitis causing total blindness. (L. P. Doyle, Jour. A.V.M.A.)

tures of the disease can be demonstrated only by histologic methods. Both eyes and brain should be examined. The iris is most frequently affected and shows mononuclear infiltrates usually consisting of small round cells occasionally mixed with large polyblastlike cells (Fig. 19.11). Exclusive infiltration with heterophils is not characteristic, and raises the question of traumatic ophthalmitis. The anterior chamber of the eye may contain granular or amorphous material (Fig. 19.12); actual cellular exudate is rare. The bulbar lesions may extend into the eye muscles, especially the *rectus lateralis* and *ciliaris*. In a comparatively small number of cases, lesions
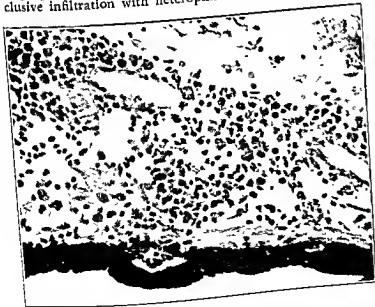
FIG. 19.11 — Ocular lymphomatosis. Experimental case. Iris showing large- and small-cell lymphocytic infiltration. (Jungherr, Storrs Agr. Exper. Sta.)

hepato-lymphomatosis (Furth, 1935), lymphosarcoma, lymphocytomatosis, certain endothelioma (Furth, 1933), lymphosarcomatosis (Burmester, 1957a), inflammatory type, Marek's disease, neoplastic type, lymphoid leukosis (Campbell, 1961).

The difficulties in defining the term visceral lymphomatosis have been discussed. Papers on fowl paralysis by Oakley (1935), Dalling and Warrack (1936), Harriss et al. (1947), Gildow et al. (1940), and others seem to have used the term lymphomatosis in the present sense. A conference of research workers (Burmester et al., 1959c) considered visceral lymphomatosis to be a broad term for neoplasia of the lymphocytic series, with lymphoblastomatosis and lymphocytomatosis to express respective degrees of cellular differentiation.

Occurrence. There hardly can be any doubt that under the conditions of modern intensive poultry production, visceral lymphomatosis represents the most common type of avian neoplasia. Highly instructive epizootiologic data have been presented by Blaxland (1956) and Chubb and Gordon (1957). In the average flock the occurrence goes parallel with the neural and ocular forms. However, one gains the impression that the visceral form occurs somewhat later than the nerve or eye type, especially during the laying period of pullets. The malady has been recognized as early as the fourth week of the brooding period. The extreme affections of the liver, so-called hepato-lymphomatosis (Furth, 1935) seem to be reserved for mature pullets or hens.

Of great interest is the increased incidence of visceral lymphomatosis in broilers, observed by Benton and Cover (1957). A recent survey of the disease at processing by Benton et al. (1962) in broilers from Delmarva and Georgia brought out the extreme variations from flock to flock both in total incidence and in the gross lesions presented, with skin and visceral lesions predominant in the female, and osteopetrosis in the male. The occurrence of the disease in turkeys is well established by the reports

of Simpson et al. (1957) and Newberne and Vosbrink (1958). Experimental propagation has been possible by cellular transplants (Belding and Sanger, 1961).

Symptomatology. The outward signs of the disease are indefinite. The comb becomes pale and shrivelled, occasionally darkened to the point of cyanosis in liver involvement. The signs may be accompanied by loss of appetite, loss of flesh, or diarrhea. Widespread attack of the mesentery can result in secondary ascites ("abdominal dropsy") and cause changes in contour and posture, spoken of as "penguin position." In systematic culling, sufficiently thin birds permit palpation of certain internal organs; thus, enlargement of the liver may be recognized by its projection beyond the metasternum and caudal margin of the ribs. The condition is often of long standing, but the observed clinical course may be quite short in that seemingly healthy birds succumb within a few days.

Pathology. Probably no other disease of birds presents a greater variety of gross pathologic pictures than visceral lymphomatosis. The large abdominal glands, such as liver and kidney, are principally affected, but there is really no organ of the body, including the skin, which is not implicated at times.

In a consideration of the gross alterations, one might commence with the spleen, because this organ is characteristically enlarged, in most cases, up to three times the normal size. Splenic hyperplasia, however, may terminate in exhaustion and atrophy. The affected spleen is usually of a grayish-brown color associated with milky thickenings in the capsule. Cross section exhibits minute grayish areas which correspond to hyperplastic lymphocytic aggregates. Instead of such diffuse changes, the spleen may present circumscribed projecting grayish tumors.

In the liver, lymphomatosis likewise manifests itself in a "diffuse" and a "nodular" variety, according to Feldman (1932). Both may be present in the same specimen. In the former variety, which is the more

common, the liver is enlarged to various degrees, grayish, and of granular surface (Fig. 19.13). The gray background is often relieved by red lines or dots of congested vessels which give the liver a characteristically *marbleized appearance*. In the nodular variety there is less enlargement, but the parenchyma is studded with firm grayish tumors of various sizes, which, unless confluent, are spherical except for surface flattening. On section these tumors are firm, smooth, lardaceous, and rarely show necrobiotic changes.

Implication of the gonads, especially in the female, has been stressed repeatedly (Pappenheimer et al., 1926). The immature ovary may show nothing more than diffuse coarse granular hyperplasia, while the producing organ exhibits alternating normal and tumorous egg follicles.

FIG. 19.13 — Visceral lymphomatosis, diffuse variety. The liver is markedly enlarged, grayish, and of granular surface. (Iowa State University.)

Extensive affection is indicated by cauliflowerlike tumors with multiple pedunculation. That such ovarian involvement may proceed at a rapid pace is indicated by birds which were observed to have a monthly trap nest record of twenty-four eggs one month prior to death from ovarian lymphomatosis. Male gonads are sometimes subject to lymphomatous affection, the disease having been diagnosed as early as the fourth week of age. Marked differences in the size of the testicles should arouse suspicion of the disease, which can be confirmed microscopically. Veritable tumor formation in the testicles is rare.

Among other internal organs the kidneys are often involved, primarily by diffuse grayish enlargement of some of the three major lobes, although nodular tumor formations occur at times. Due to the decrease of the renal secretory surface, affected kidneys may show secondary nephritic changes of the nonaffected lobes. Without concomitant lymphomatous alterations, these kidney lesions may be difficult to differentiate from uremic nephritis (visceral gout).

The heart shows gross changes more *frequently than is realized*; there occur either grayish striations (tigering) in the epicardium, or prominent myocardial tumors which lead to deformities and local adhesive pericarditis; at times, the bicuspid valve presents small tumorous nodules. The lymphomatous process is also apt to originate in or spread to adjacent organs such as the proventriculus, gizzard, and lungs (Fig. 19.14). Multiple tumors are often implanted in the mesentery and peritoneum, recalling the appearance of "pearl disease" in bovine tuberculosis. In such cases there is apt to be serous transudation into the abdominal cavity. Oviduct and mesosalpinx are comparatively refractory.

The glands of the head and the skin are subject to attack by the process. Unilateral swelling of the cheek, as in "roup," may be due to lymphomatosis of the nasolacrimal glands. Affected integument exhibits small multiple tumors of the feather

FIG. 19.14 — Visceral lymphomatosis. Marked tumorous involvement of both lungs. (F. D. Patterson, Iowa State University.)

follicles or large skin tumors prone to superficial ulceration. Skin involvement often escapes detection until the birds are dressed. Helmboldt *et al.* (1963) studied 44 cases of skin leukosis in condemned 9-week-old broilers and found the majority to be associated with brain, spinal cord, and peripheral nerve lesions, in falling order. Some of the controls also presented brain lesions.

The condition of the bone marrow is ordinarily analogous to that described in neural lymphomatosis (Johnson, 1934), but frank leukemic cases of lymphomatosis may show minute grayish tumors (Furth, 1933).

Endothelioma apparently is a rare type of tumor in visceral lymphomatosis. It was first described as a transmissible condition in the fowl by Begg (1927), and again observed in transmission experiments of lymphomatosis by Furth (1933), Jungherr (1937), and Burmester (1957b). Gross combinations of edematous grayish and hemorrhagic tumors should make one suspect an endotheliomatous process; the specific diagnosis rests upon microscopic study.

In contrast to the gross manifestations, the histopathologic picture of visceral lymphomatosis is comparatively uniform. Typical changes are represented by massive accumulations in various organs of proliferating lymphoid cells which are usually much more widely distributed than is suggested by gross alterations. The

FIG. 19.15 – Visceral lymphomatosis. Section of liver showing massive lymphoid infiltration. (Pappenheimer, Storrs Agr. Exper. Sta.)
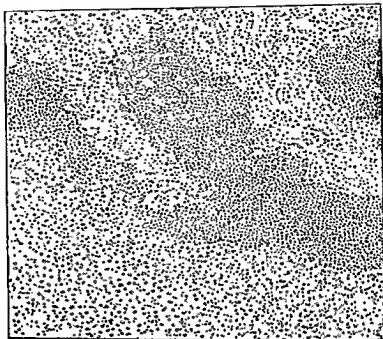
pathologic elements are represented by two prototypes, namely, so-called small round cells resembling lymphocytes, and large lymphoblastlike cells. Between these extremes lymphoma types occur. The small more mature cell type is apt to occur in visceral complications of the neural form and in the extreme cases of liver enlargement (Fig. 19.15), while the large cell type prevails in rapidly growing lymphoid tumors.

Difficulties may arise in the interpretation of microscopic lymphomatous changes. As would be expected, the lesions have a predilection for organs containing normal lymphoid tissue. With the exception of thymus, cecal tonsils, bursa of Fabricius, submucosa of the intestine and of the secondary bronchi, there are no organized lymph nodes in the fowl; but microscopic lymphoid foci are usually present without anatomic regularity in the parenchymatous organs and the digestive tract. These follicles are subject to stimulation, aside from the etiologic agent of lymphomatosis, also by other viral factors (e.g., avian encephalomyelitis). Thus it is

often difficult to state whether a given lymphoid follicle represents normal, reactive, or neoplastic tissue. A guide to interpretation should come from consideration of all the pathologic evidence available in the case. In ordinary fixed tissues the large lymphoblastlike cells are difficult to differentiate from myeloblasts, hemocytoblasts, etc. Special fixing and staining methods designed to bring out the differential characters of hematopoietic elements are of value. Recourse may be made to impression smears or "Klatsch" preparations stained according to Wright and/or Giemsa.

In a series of six detailed papers Lucas and his associates (1949, 1950, 1951) have studied the relation of so-called normal lymphoid foci in the pancreas to lymphomatosis in chicken, turkey, dove, pheasant, wild mallard, and White Pekin duck. They concluded that the amount of ectopic lymphoid tissue was approximately proportional to the predisposition toward lymphomatosis. While the question could not be answered definitely whether these lymphoid foci were actually induced or

only stimulated by the lymphomatosis agent, the report of the United States Bureau of Animal Industry Regional Poultry Research Laboratory (1951) suggested the latter.

A major contribution to the question of lymphoid tissue in chickens on purely anatomical grounds was made by Biggs (1957). He reviewed the work of Kondo (1937) in Japan and confirmed the concept that noncapsulated mural lymphoid nodules occur normally along the lymph vessels which accompany the lymph veins. Dennington and Lucas (1960) reduced significantly the number of ectopic lymphoid foci in the pancreas by intermittent heat treatment during the first 16 days of brooding. They cited these findings in support of the hypothesis that such lymphoid structures are not ontogenetically normal, but the result of a defense reaction.

Regarding the histopathology of individual organs, the spleen in early cases exhibits lymphoid hyperplasia around the "adenoid sheaths," which are the functional equivalents of the Malpighian corpuscles in mammals. The hyperplastic areas become confluent and form broad anastomosing masses in the red pulp. True Malpighian corpuscles are absent in the bird, but organized lymphoid follicles appear irregularly in the normal spleen.

Campbell (1956) claimed differentiation of inflammatory lymphomatosis and neoplastic lymphoid leukosis, in his terminology, by histopathologic means. Similar changes, however, are seen under normal and reactive conditions (Thorbecke *et al.*, 1957).

The liver is often involved in the absence of gross lesions. Miliary lymphoid accumulations appear in the parenchyma or the portal islands. In the latter location, as elsewhere, there is frequently a secondary admixture of metamyelocytes and heterophils, probably because such areas are still endowed with some of their embryonic hematopoietic potentialities. For severe cases massive compact expanses of lymphoid tissue are typical. They are in sharp relief from the surviving liver tissue

by virtue of their basophilic staining affinity. The hepatic parenchyma itself shows only slight damage, a fact which may account for the surprising vitality of some birds in the presence of liver involvement. Horiuchi (1961) recognized the following three histologic types: the nodular one, characterized by fibroid cells which are continuous with the sinusoidal epithelium; the diffuse type in which cellular proliferation takes place along the spaces of Disse; and the infiltrative type which consists primarily of a network of sinusoidal endothelium.

The ovary shows either follicular or infiltrative lymphoid alterations; the latter often encroach upon or engulf ovarian follicles. In the kidney the process exhibits frequently an infiltrative interstitial character; since "interstitial nephritis" is rare in birds, such changes are often an expression of lymphomatosis. The myocardium is particularly susceptible; in the beginning the lesions are infiltrative but later form coherent neoplastic masses. The cardiac muscle of healthy young birds often shows small ectopic foci of lympho- and granulopoiesis (Pappenheimer *et al.* 1929a). Although recognized now as part of the normal histoanatomy of the chicken, it may be these foci which undergo neoplastic degeneration under the influence of the lymphomatosis agent. Sclerosis of the coronary arteries is common in mature birds, but there is an approximate six-fold increase of incidence in chickens exposed to the lymphomatosis agent according to Paterson and Cottral (1950).

Endothelioma is considered to be a possible extension of the microscopic features of lymphomatosis. According to Furth (1934), neoplastic growth of endothelium may occur in any location of normal endothelium, including the liver, spleen, and bone marrow. Quite often these tumors are of purely microscopic size. The architecture is variable and may present solid, glandular, syncytial, and angiomatous types. The tumor is characteristically composed of basophilic cells with large chromatin-poor vesicular nuclei which ex-

FIG. 19.16 — Endothelioma in visceral lymphomatosis. Experimental case. Section of muscle affected with giant-cell endothelioma. (Jungherr, Storrs Agr. Exper. Sta.)
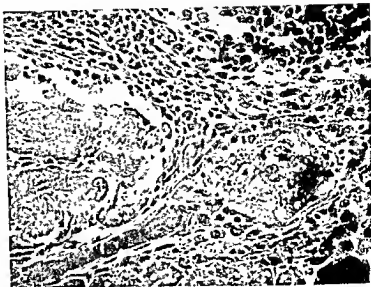
hibit thick nuclear membranes and prominent nucleoli. There frequently occur groups of multinucleated giant cells not unlike the Langhans type in tuberculosis (Fig. 19.16). The tendency toward necrobiotic changes is marked. The diagnosis of endothelioma is strengthened by demonstrating connection with normal endothelium. However, it is often difficult to decide whether the tumor is of endothelial, mesenchymal, or mesothelial origin.

Hematology. Much of what has been stated under this heading in regard to neural lymphomatosis is applicable to the aleukemic cases of visceral lymphomatosis. In other instances the blood picture may show subleukemic or leukemic lymphoid alterations. The term "subleukemic" is used, in accordance with the suggestion of Furth (1933), to indicate a moderate increase of the leukocyte count, up to 100,000 per cu. mm. of blood.

Specific blood changes are either qualitative or quantitative in nature, of which the former deserve special attention; they are often transitory or terminal and, on the whole, less constant than in erythro- and granuloblastosis. Aside from the appearance of "budded" or pseudopoded lymphocytes in increased number, the blood picture may be characterized by the presence of immature lymphocytes in the circulating blood. The degree of immaturity of the leukemic cell varies. According to Furth (1933), in subleukemic forms one observes primarily medium-sized basophilic lymphocytes showing vacuoles and azurophilic granules. The large lymphocyte predominates in frank leukemic cases. Apart from size, these cells are distinguished by a large eccentric nucleus composed of spongy chromatin and a comparatively narrow mass of intensely basophilic cytoplasm. These cells are considered by Furth (1934) to be capable of producing erythroblasts, myelocytes, and lymphocytes and thus correspond to the hemocytoblast in the terminology of Ferrata-Maximow. The lympholeukemic alterations may be accompanied by anemic changes, such as basophilic erythrocytes and erythroblasts; also erythrogonia suggestive of erythroblastosis occur occasionally.

Differential diagnosis. Of other diseases capable of forming tumorlike nodules in the visceral organs, tuberculosis and pullorum disease must be kept in mind. Avian tuberculosis also attacks primarily spleen and liver, but the nodules are usually yellowish, granular on the surface, and readily separable from the parenchyma of the affected organ. Pullorum nodules are

found in the heart of adult birds, particularly males, but as a rule are accompanied by inflammatory and congestive phenomena. In both diseases bacterioscopic and cultural tests can give further clues to the etiologic nature of the pathologic processes.

In turkeys, low grade lesions of histomoniasis in liver and spleen as seen on the inspection line, are suggestive of visceral lymphomatosis but may be diagnosed histologically by their granulomatous character (McKee et al., 1963).

OSTEOPETROSIS

Synonyms. Thick-leg disease, marble bone, akropachia ossea and hyperplastic ostitis (Reinhardt, 1930), hypertrophic osteitis (Reis and Nobrega, 1936), osteodystrophia fibrosa cystica (Gohs, 1934a, b), diffuse osteoperiostitis (Pugh, 1927), Paget's disease (Venkataraman, 1936), osteopetrosis gallinarum (Jungherr and Landauer, 1938).

Hypertrophic osteopathies of the fowl have received occasional mention in the literature (Reinhardt, 1930). Sporadic outbreaks of diffuse osteoperiostitis in male birds have been reported by Pugh (1927) in England, and Venkataraman (1936) in India. Brochet (1935) named several diseases as possibly leading to bone deformities, namely acromegaly, Paget's disease, tuberculosis, gigantism, osteosarcoma and hypervitaminosis; he thought hypertrophic bone changes in birds to be secondary to respiratory infections or endocrine dysfunctions. Campbell (1954) and Hutt and Cole (1954) maintained that the condition should not be included in the complex under discussion.

Experimental studies along this line are of special interest. Oberling and his associates (1938c, 1934) maintained birds in outdoor cages on a mineral-poor but otherwise adequate diet and observed lesions which showed a striking resemblance to human osteodystrophia fibrosa cystica associated with parathyroid hyperplasia. Gohs (1934a, b), on the other hand, produced a similar pathologic picture, save

for parathyroid hyperplasia, together with leukemic conditions, by repeated injections of normal embryonic or X-rayed adult avian bone marrow. Spontaneous cases pathologically resembling those of Gohs were described under the term osteopetrosis gallinarum (Jungherr and Landauer, 1938) and found to be transmissible. The agent could not be separated from that of lymphomatosis. While confirming the associated occurrence, Brandly et al. (1941) observed certain differences in occurrence, and Burmester (1947a), in sedimentability, between osteopetrosis and lymphomatosis which, according to them, suggest etiologic differences. Periosteal proliferation in long bones of chicken embryos receiving intravenous injections of normal blood or serum was observed by Brandly (1941) and his associates (1949). The condition described by Thiersch (1944) under the name of osteopathia hyperostotica scleroticans multiplex infantilis, following intravenous injection of embryos with human leukemia material, seems to be of similar nonspecific character.

Osteopetrosis differs from osteomyosclerosis, described in fowl by Seifried and Sassenhof (1940), as a disease sui generis, and is believed by Bloom et al. (1941) and Sjolte (1950) to represent a physiologic mechanism for calcium deposition during egg production.

Occurrence. In comparison with other manifestations of the avian leukosis complex, the occurrence of osteopetrosis is rare. Some field cases are sporadic in nature and often escape detection until the birds are dressed. This is borne out by observations of the United States poultry meat inspection service. Isolated epiornithic cases, predominantly in males, have been described. The geographic distribution appears to be widespread according to reports from Canada (Moynihan, 1943; Biely, 1943); Sweden (Magnusson, 1946); and South Africa (Coles and Bronkhorst, 1946). In a flock kept for experimental purposes, the latter authors observed 39 cases which were believed to show a famil-
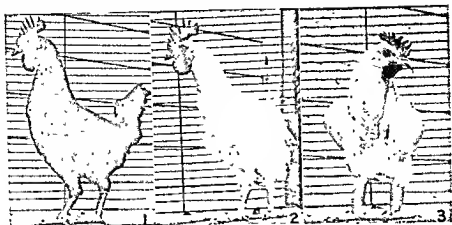
FIG. 19.17—Osteopetrosis. The shanks of the birds are affected in various degrees. (Jungherr and Landauer, Storrs Agr. Exper. Sta.)

ial incidence and to be due to a unifactorial recessive character, in support of the contention of Hutt (1932), who found two of nine birds from the same dam to be affected with such abnormal osteogenesis.

Symptomatology. If the disease affects the metatarsi it can be detected on clinical inspection. Palpation of the long bones may reveal additional cases. In the beginning, diseased leg bones, to the exclusion of the phalanges, show abnormal convexities or irregular thickenings in the diaphyseal or metaphyseal regions. The affected areas are hot to the touch, hard, and insensitive. Advanced cases exhibit the characteristic "bootlike" thickening of the shanks (Fig. 19.17).

Pathology. The gross alterations of the skeleton may be observed in all the long bones of the extremities (Figs. 19.18 and 19.19), in the osseous components of the pelvis, shoulder girdle, and in rare instances also in the spine, while phalanges and skull bones seem refractory. X ray pictures examined by Edeiken (1940) were considered to show definite bone changes in the nature of osteosclerosis with thickening and increase in density of the cortex, encroachment and in some places obliteration of the medullary cavity (Fig. 19.19); the general features were regarded as similar to those of osteopetrosis in man.

The pathologic process affects primarily

the diaphysis, and is ordinarily bilateral. The intensity of the bone lesions varies widely from exostosislike cortical thickenings to massive asymmetrical involvement leading to almost complete obliteration of the marrow cavity. Even in the early stages the affected bones—though somewhat porous in appearance—give evidence of increased breaking strength. In cases of long standing they are extremely hard. Both cross (Fig. 19.20) and longitudinal sections of bones are helpful in the diagnosis.

The extent of the visceral changes is variable. Rapidly developing cases usually exhibit the gross characteristic changes of visceral lymphomatosis, while old arrested cases are more apt to show this association only on microscopic study of the tissues. The parathyroids appear normal.

The histopathologic picture of affected bones varies according to the maturation of the specific process. The initial phase is characterized by sequestration and granular degeneration of old trabeculae, marrow fibrosis, and increased osteoclasia. These changes, akin to those of osteodystrophia fibrosa, are accompanied by the development of a new large-celled vascular fibrous bone tissue which fails to show cartilage remnants or other evidence of endochondral ossification (Fig. 19.21). In the florid phase the new bone tissue gradually replaces both the original spongiosa and
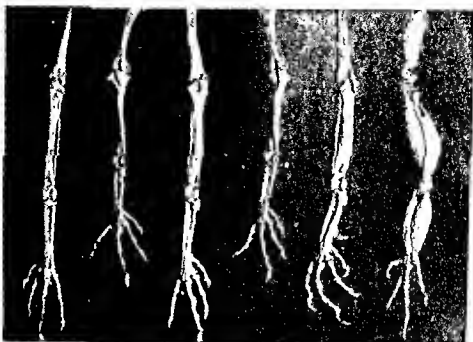
FIG. 19.18 — Osteopetrosis. Macerated leg bones affected in various degrees. First on left, normal. (Jungherr and Landauer, Storrs Agr. Exper. Sta.)

FIG. 19.19 — Osteopetrosis. X-ray picture of a White Rock chick about 9 weeks old; almost all long bones were involved. (Vineland Poultry Laboratories, Vineland, N.J.)
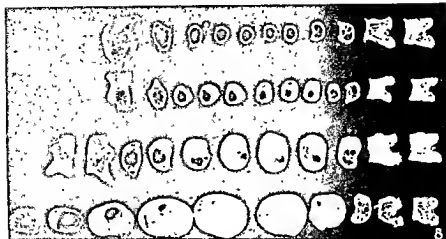
FIG. 19.20 — Osteopetrosis. Cross section of affected femora. Upper row normal. (Jungherr and Landauer, Storrs Agr. Exper. Sta.)

FIG. 19.21 — Osteopetrosis. Section of meta-tarsus showing initial lesions. (Jungherr and Landauer, Storrs Agr. Exper. Sta.)

compacta, while osteoclastic activity regresses. In the arrested phase, the new bone lamellae appear condensed, hypercalcified, and subdivided by numerous thick irregular cement lines (in formalin-fixed material) corresponding to the so-called "mosaic" structure of Paget's dis-

ease in man (osteitis deformans) (Fig. 19.22). All three phases may be observed in the same case or even the same bone section. Gross et al. (1959) observed an early increase in size and number of periosteal and endosteal cells which process they considered neoplastic, especially be-

FIG. 19.22 — Osteopetrosis. Section of meta-tarsus showing arrested lesions with "mosaic" structure. (Jungherr and Landauer, Storrs Agr. Exper. Sta.)

cause it was often accompanied by osteolysis (Swiss cheeselike). The later formation of imperfect lamellar bone they considered nonneoplastic. Campbell (1961) considered osteopetrosis as a virus-induced bone deformity caused by increase of osteoblastic activity. This may be associated with immaturity of gonads, atrophy of spleen, adenomas of kidney, tumors of liver, and hyperplasia of certain muscle groups (Campbell, 1963).

Hematology. The blood picture is ordinarily aleukemic. There is sometimes a relative or an absolute lymphocytosis. Evidence of secondary anemia is quite common and understandable in view of the progressive reduction of the hematopoietic tissue. The remaining bone marrow is intensively hyperplastic and on microscopic scrutiny shows basophil erythroblasts and erythrogonia, as in erythroblastosis. In spite of this hyperactivity of the marrow, the immature stages of erythropoiesis are not observed in the circulating blood.

Differential diagnosis. Among other avian osteopathies, rachitis and osteoporosis (rickets) can be differentiated from osteopetrosis by their epiphyseal formation of osteoid or porous bone tissue, accompanied in osteoporosis by parathyroid hyperplasia. Cases of osteopetrosis complicated by D-avitaminoses occur. In perosis (hock disease) there is twisting and flattening of the shanks, while the bone structure itself remains normal.

NEPHROBLASTOMA

Synonyms. Renal adenocarcinoma (Carr, 1956), embryonal nephroma, Wilm's tumor, nephroblastoma (Ishiguro et al., 1962; Walter et al., 1962).

The addition of this tumor as a patho-

FIG. 19.23 — Nephroblastoma in chicken. Dilated tubules with neoplastic epithelial lining. Sarcomatous degeneration of stromal connective tissue at margins. (Courtesy of Dr. T. N. Fredrickson, U.S. Regional Poultry Laboratory, East Lansing, Michigan.)

logic entity to the avian leukosis complex has been mentioned in the historical part. Although always considered rare, this tumor is now found routinely in birds subjected to condemnation on the processing line, according to G. S. McKee (Northeastern Avian Diseases Conference, 1963).

Gross focal tumors in the kidney may suggest such a diagnosis which must be verified histologically. The microscopic picture varies widely from simple hyperplastic tubules and attempts to form glomeruli (Fig. 19.23) to frank sarcomatous stroma. There may be islands of cartilaginous, osteoid tissue and epithelial pearls, all of which give the impression of a teratoid tumor. Metastases in field specimens are rare.

In uncomplicated cases the blood picture is normal. Histologic examination permits differential diagnosis from other conditions causing enlargement of the kidneys, such as visceral lymphomatosis and uric nephritis.

ERYTHROBLASTOSIS

Synonyms. Severe anemia, "light," intravascular lymphoid leukosis (Ellermann, 1921), oligoerythrocythaemia or erythromyelosis (Bayon, 1929, 1930), erythroleukosis (Ellermann, 1923 and Furth, 1931b), Leukomyelose (Kitt, 1931), the erythroblastic form of transmissible fowl leukosis (Olson, 1936), erythroblastosis (Engelbreth-Holm and Rothe-Meyer, 1932).

Following the initial report by Ellermann and Bang in 1908 and subsequent studies (1921, 1923) on the experimental production of fowl leukemia by parenteral transmission of blood or blood-forming tissues, only Schmeisser (1915) reported on the successful transmission of a spontaneous case. As doubts had thus arisen in regard to the transmissibility of fowl leukosis, Jármai (1930) and Furth (1931a) independently developed new transmissible strains of fowl leukosis which proved to be of the erythroblastic and erythrogranuloblastic type, respectively. These results were soon confirmed by Engelbreth-Holm

(1931) in Denmark, and Oberling et al. (1933) in France, and others. From the pathologic standpoint most investigators recognized an erythroblastic and a granuloblastic (myeloid) type. Since both of them occurred in subpassages either alone or together, irrespective of the disease in the donor, they were considered to be due to the same etiologic agent.

In transmission experiments on erythroleukosis, Ellermann (1923) observed severe cases of anemia and regarded them as hyperacute aleukemic expressions of the disease. Severe spontaneous anemia of fowl associated with lipochromatosis was considered to be different from Ellermann's erythroleukosis by Bedson and Knight (1924). By transmission of such cases, however, Stubbs and Furth (1932) were able to show their close relationship to erythroleukosis. Therefore, most investigators recognize that erythroblastosis may occur in a leukemic and an anemic subvariety (Olson, 1940).

Occurrence. In contrast to the amount of experimental work that has been conducted with it, erythroblastosis is of comparatively rare sporadic occurrence under field conditions. It is known to affect all standard breeds and occurs primarily after the age of six months (Olson, 1936). A notable exception is the report of Hamilton and Sawyer (1939), who observed the disease in five-week-old birds on an epiornithic scale.

Symptomatology. While birds in the early stages of erythroblastosis appear normal, within a short time the disease manifests itself by paleness or by a yellowish color of the unfeathered parts of the body, and by stupor and diarrhea; there is usually emaciation and loss of egg production. Uncontrollable bleeding from feather follicles has been observed at times. The anemic subvariety takes a chronic course over a period of several months.

Pathology. The carcass may be emaciated, at times obese. Although the tissues appear pale, there are often petechial hemorrhages in various places such as the mucosa of the small intestine, under the liver

capsule, or in the subcutaneous tissue. Terminally there is sometimes evidence of thrombosis, infarction, and rupture of internal organs. Edematous changes prevail in chronic cases. The most typical gross alteration is a diffuse enlargement of liver and kidneys and especially the spleen, associated with a cherry-red discoloration in fresh specimens. The parenchymatous organs are soft and friable. In uncomplicated cases there is no tendency toward the production of deforming tumors. Some of these changes may be absent, and even if they are well developed they are only suggestive and not diagnostic of the disease. Of special interest is the appearance, in normally lipo-pneumatic long bones, of the marrow which shows marked hemorrhagic hyperplasia and increased consistency described as "currant-jelly-like." The compacta of the long bones may undergo osteosclerotic changes (Bayon, 1930; Furth, 1931a).

In anemic cases of erythroblastosis the hyperplasia of the visceral organs and the bone marrow is absent; the spleen occasionally may be in a state of atrophy. The marrow spaces are often replaced to a large extent by a honeycombed spongy bone formation such as is seen in osteodystrophia fibrosa (Stubbs and Furth, 1932). Whether or not a given case of severe chronic anemia (asthenia in older textbooks) is causally related to erythroblastosis can be ascertained only by passage experiments.

On histologic examination, the lesions of erythroblastosis, aside from abnormal deposits of hemoglobin-derived pigments (Bayon, 1930), are characterized by dilatation of the blood sinusoids and capillaries which are filled to capacity with primitive blood cells of the erythroblastic series. This essentially intravascular process is called leukostasis (Furth, 1931b) and is particularly developed in the liver, spleen, and bone marrow. In studying the pathogenesis of the disease in the bone marrow, Pontén and Thorell (1957) found the sinusoids to show focal involvement within 3 days and diffuse involve-

ment within 7 days postinoculation. Nonspecific regeneration, following the use of hemolytic chemicals, commenced within 12 hours and was diffuse or "systemic" in character. Since mild hematologic changes suggestive of erythroblastosis may occur in other conditions, leukostasis is one of the prime features in the diagnosis of erythroblastosis.

In anemic erythroblastosis the visceral organs, especially the liver, often show extensive accumulations of small round cells and granulocytes which are probably reactive in character and difficult of interpretation. On careful search one is sometimes able to detect typical localized areas of leukostasis (Stubbs and Furth, 1932). According to Engelbreth-Holm (1932), the bone marrow quite regularly shows microscopic accumulations of erythrogonia which, however, fail to invade the blood stream.

Hematology. Even on gross examination the blood is often noticed to be pale, watery, and slow to clot. As shown by Furth (1931a), centrifugalized specimens of fresh normal blood show a plasma:leukocyte:erythrocyte ratio of about 55:1:44, erythroblastic blood of 88:1:11, and granuloblastic blood of 16:69:15. Centrifugation of fresh blood in chilled tubes may thus serve as a presumptive diagnostic test, and in the case of erythroblastosis also gives evidence of severe anemia. In properly stained smears the blood picture is characterized by the appearance of many basophile erythroblasts and erythrogonia, which are considered to be hemoglobin-free precursors of erythrocytes. Although these cells are not strictly leukocytes, they would show up as leukocytes by the ordinary leukocyte count technique. The number of immature erythrocytes in the circulating blood is relatively small, a few hundred thousand per cu. mm. (Furth, 1931b). The erythrogonia—which have been given the confusing name of lymphoidocytes by Ellermann—are highly characteristic; they vary in size, but are usually larger than the erythroblasts. In contrast to the "cartwheel-like" nucleus of

these cells, the nucleus of erythrogonia stains a peculiar violet-red (in Wright-Giemsa preparations), and appears either homogeneous or diffusely hromatic. Binucleated and mitotic figures occur among them. The cytoplasm appears narrow, basophilic, and occasionally vacuolated (colored illustrations, Furth, 1931b, and Oberling and Guérin, 1934). There is often an intense thrombocytopenia (Feldman and Olson, 1933), which may be due to a concomitant proliferation of the corresponding immature precursors, a thromboblastosis (Ishitani, 1957). The number of polychrome erythrocytes is comparatively small. This is in contrast to the situation in secondary anemias — which may occur spontaneously or be induced by repeated bleeding or by certain chemicals — in which polychrome erythrocytes together with anisocytosis and poikilocytosis dominate the blood picture (Furth, 1931b).

Differential diagnosis. The danger lies not so much in mistaking erythroblastosis for another disease as in not suspecting it if it is present in an atypical or chronic form. Confirmation of the diagnosis must rest upon hematologic and histologic studies.

MYELOBLASTOSIS

Synonyms. Leukemic myeloid or myelotic leukosis (Ellermann, 1922), Leukomyelose (Kitt, 1931), leucocythemia or leucomyelosis (Bayon, 1930), leukemic myeloblastosis (Nyfeldt, 1934), the granuloblastic form of transmissible fowl leukosis (Olson, 1936), granuloblastosis (Jungherr et al., 1941), myeloblastosis (Eckert et al., 1951), diffuse myeloid leukosis (Darcel, 1957).

As has been emphasized by Furth (1934), the description of leukemic and aleukemic types of myeloid leukosis by Ellermann (1920) comprised two pathologically different conditions, namely myeloblastomatosis, which is identical with myeloblastosis, and myelocytomatosis. Aside from this, the historical development of available knowledge on myeloblastosis and erythroblastosis has been much the same, due to tendency of these diseases to occur in a mixed form (Furth, 1931a). Nyfeldt (1934) claimed to have observed a pure strain producing only myeloblastosis. The development of a pure strain (BAI-A) of myeloblastosis (Beard, 1956), although later shown to have multiple cell responses, has been mentioned in the historical part.

Occurrence and symptomatology. There are no essential differences between myeloblastosis and erythroblastosis. Bayon (1930) believes that the former occurs mostly in old hens.

Pathology. In distinction from erythroblastosis, the disease has a tendency to bring about grayish mottling of the enlarged parenchymatous organs; the bone marrow appears "pale or pink and diffluent" (Bayon, 1930). The liver may have a granular or "morocco-leather" appearance (Chubb and Gordon, 1957). Otherwise, the anatomic changes are those described under erythroblastosis. Gross differential diagnosis between the two types is usually not possible (Olson, 1936). The histopathology of predominantly myeloblastic cases shows massive accumulation in the parenchymatous tissues of large myeloblastic and promyelocytic elements which are essentially extravascular, but overflow into the blood channels. There is therefore marked infiltration and substitution of the original tissues by the pathologic cells, in distinction from the fairly uniform intravascular leukostasis in erythroblastosis.

In the spleen the early changes seem to begin in the reticular stroma of the red pulp. The bone marrow shows intense myeloblastic activity in the extrasinusoidal areas, an observation which is particularly useful in pathogenetic studies. As may be surmised, the respective pathologic and hematologic features of erythro- and myeloblastosis show considerable overlapping in the mixed form.

Lagerlof and Sundelin (1963a) studied the histogenesis of the induced disease and found it to be similar to that of erythroblastosis (Pontén and Thorell, 1957) in that the bone marrow was attacked within
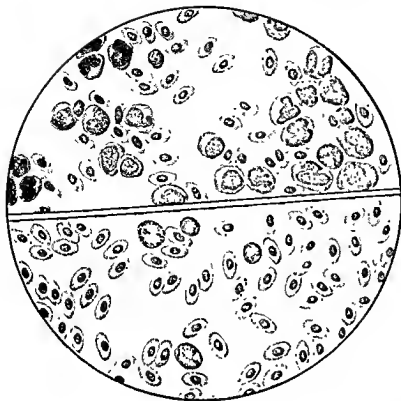
FIG. 19.24 — Comparison of blood smears from (above) myeloblastosis and (below) neural lymphomatosis. (Pappenheimer, Storrs Agr. Exper. Sta.)

3 to 4 days, but by the formation of multiple extrasinusoidal foci. Newly hatched chicks, inoculated with the virus in plasma samples, developed the "classic" form. Susceptibility decreased with age and some chicks developed subleukemic hemolytic anemia with lymphoid hyperplasia, splenomegaly, and splenic siderosis. Chicks inoculated at 14 to 21 days of age showed cases of visceral lymphomatosis, teratoid tumors, and hemangiomatosis (Lagerlöf and Sundelin, 1963b).

Hematology. The marked rise in the leukocyte column of centrifuged blood specimens has been mentioned (p. 419). The blood picture is characterized by the appearance of primitive cells of the myeloblastic series, especially myeloblasts (Fig. 19.21) and promyelocytes (colored illustrations, Oberling and Guérin, 1934) in large numbers, up to 2 million per cu. mm. (Beard, 1956), indicative of a leukemic or subleukemic state. The myeloblasts are large cells with slightly baso-

philic clear cytoplasm and a large vesicular acidophilic nucleus containing one to four nucleoli. Occasionally the leukemic elements are of the "Rieder" cell type (Olson, 1936). Promyelocytes and myelocytes have a similar nuclear structure, but can be definitely recognized by virtue of their specific granulation, which is primarily basophilic in the early forms, or grades into that of the three familiar types of adult granulocytes. The peroxidase test, useful in mammalian hematology for the recognition of the granuloblastic series, does not give equally sharp results with avian blood, according to Jover (1951). Hemocytoblasts are indistinguishable from myeloblasts; if they occur together with definitely granulated elements, the former term seems to be more confusing than clarifying. In addition to the blood alterations mentioned, there may be evidence of secondary anemia. Late myelocytes and metamyelocytes with the granulation of the mature heterophil are rare.

MYELOCYTOMATOSIS

Synonyms. Aleukemic myeloid leukosis (Ellermann, 1920), leukochloroma (Mathews, 1929), myelocytoma (Pentimalli, 1915; Feldman, 1932), myelocytomatosis (Furth, 1933), aleukemic myeloblastosis (Nyfeldt, 1934), myeloma, discrete myeloid leukosis (Darcel, 1957).

While it is difficult to state the exact nature of the disease described by Ellermann as aleukemic myelosis, Pentimalli (1915) first recognized the distinctive character of the tumor found in this disease, which Mathews (1929) described as an essentially aleukemic neoplasia. In passage experiments of lymphomatosis (strain 2) Furth (1933) observed leukemic cases of myelocytomatosis and considered both to be pathologic expressions of the same agent. "Pure" transmissible strains, with the possible exception of Nyfeldt's strain (1934), have apparently not been described in the literature. According to the available experimental evidence, myelocytomatosis is thus related to lymphomatosis, while everyday pathologic experience tends to place it closer to the ordinary tumors.

Occurrence and symptomatology. Sporadic cases are apt to occur in young adult birds, some of which are seen in flocks that do not show any other pathologic evidence of the avian leukosis complex. The clinical appearance is noncontributory to the diagnosis.

Pathology. Myelocytomatosis characteristically forms tumors which figure among the few avian representatives which can be recognized on gross examination with some degree of certainty. The new growths are a yellowish-white color, resemble congealed cream in appearance and consistency, and are often of multiple anatomic origin. The masses may be found in the muscle tissue and visceral organs, and especially along the ribs or other parts of the skeleton bordering the body cavities, and may exert pressure on the spinal cord.

Histologically, the tumors consist of compact masses of myelocytes which are strikingly uniform in appearance and have the typical full acidophile granulation of either the mature eosinophil or the heterophil. The stroma is very scanty. In certain parenchymatous organs, such as the kidneys, the tumors show infiltrative growth. The bone marrow, as a rule, exhibits myelocytic hyperplasia. The type cell is ordinarily round, but may appear fusiform in areas subject to pressure.

Hematology. Uncomplicated cases are usually aleukemic, although there may be a heterophile leukocytosis. If definite leukemic blood involvement occurs, the hematologic picture is dominated by the appearance of polychrome myelocytes and acidophilic metamyelocytes.

ETIOLOGY

Discussion of the etiologic aspects of the avian leukosis complex presents difficulties which are brought about by the lack of definite information on the causal relationship of the various forms of tumor and the relationship of the known agents to tumor induction. As is expected with a disease of almost universal occurrence and obscure etiology, the literature contains a wide variety of suggested causes. Some points are of more or less general applicability.

Most observers are in agreement with the opinion of the early investigators (Marek, 1907; Pappenheimer et al., 1926; Ellermann and Bang, 1908) that the condition is not caused by a cultivable organism of bacterial or fungal nature. Gray (1938) sometimes isolated Coccaceae from affected nerves.

On the evidence that field cases of fowl paralysis and allied conditions occur frequently in association with coccidiosis or helminthiasis, some students believe that parasitic conditions act as predisposing, precipitating, or even causal factors. Bayon (1930), for instance, observed Davainea proglottina infection in erythroblastosis and severe anemia, and considered it to be a contributory factor, while Stubbs and Furth (1932), studying the same disease, failed to observe this association. The fact that typical cases of the various diseases

of this group have been produced under controlled laboratory conditions (Warrack and Dalling, 1932) militates against a synergistic concept in the causation of the avian leukosis complex. Caranti (1956) believed cases which pathologically correspond to neural lymphomatosis to be causally related to coccidiosis. The diagnosticians observing field cases continue to suggest some relationship between coccidia and leukosis, however, the question of predisposing and aggravating factors must remain open and should best be attacked on an experimental rather than a statistical basis.

Some nutritional aspects were studied by Wilcke and his associates (1933), who found no significant differences in the incidence of fowl paralysis when the rations varied in mineral and vitamin levels. Blount (1932) interpreted this disease in terms of a B-hypervitaminosis or, more generally, of a gastronomic enteritis. On the basis of observing functional curative effects from the feeding of lettuce (Bayon, 1932), or a decrease in the incidence of the leukotic diseases following the feeding of additional wheat germ oil (Butler and Warren, 1958; together with Hammersland, 1958), some authors suggested the possibility of an underlying E-hypovitaminosis; the latter contention could not be supported by critical experimentation (Jungherr, 1910; Taylor and DeOme, 1939). Adamstone (1936), however, claimed to have produced a "lymphoblastoma"-like condition in chicks reared on a diet which had been treated with ferric chloride for the purpose of destroying vitamin E.

Biely and March (1959) studied the effect of nutrition on the level of leukosis mortality in 4 strains of chickens, 2 of which were considered susceptible. In all 4 of the strains, the incidence of tumors was lowest in those chickens raised and maintained on a low plane of nutrition as compared with the same birds on a high plane of nutrition. Olson et al. (1962) found one particular lot of cod liver oil to increase the incidence of lymphomatosis induced by exposure to low doses of viral inoculum or contact. The incidence of erythroblastosis induced with the same material in high doses was not altered.

Nonspecific factors such as Salmonella toxins, vitamins A and K and iron deficiencies, poor ventilation, etc., have been claimed by Emmel (1939) to be the basic process in the development of the avian leukosis complex. McIntosh and Selbie (1939) believed they produced filterable tumors in fowls by the injection of tar, but extended studies by Murphy and Sturm (1941a, b) and more recently by Crispins (1960) on chemically induced tumors have failed to establish transmissibility of cell-free materials. Such transmission continues to be described but not under circumstances where concurrent or subsequent virus contamination could be ruled out.

The heritability of resistance and susceptibility to death from tumors has been studied in the laboratory and through field observations.

Bayon (1932) voiced the widespread opinion that the incidence of fowl paralysis was higher in the progeny from certain strains of fowls. Asmundson and Biely (1932) and Biely et al. (1952) found differences in the resistance to fowl paralysis, which they interpreted as due to a dominant inherited factor. Hutt (1939) and his associate (1918) observed reduction from 15 to 5 per cent in adult mortality due to neoplastic diseases after twelve years of selecting disease-resistant lines. Cole (1911) developed White Leghorn resistant and susceptible lines for an artificially transmissible sarcoma (Jungherr, 1937), but found they showed no differences in spontaneous mortality which was principally due to lymphomatosis. Purchase (1963) reported preliminary results indicating that RPL line 7, resistant to infection with RPL-12 and Rous virus, is very susceptible to JM virus. This would indicate that genetic resistance to one type of neoplasia does not necessarily mean resistance to neoplastic disease in general. In ordinary observations it is difficult to differentiate between genetic susceptibility and actual transmis-

sion via the egg of the causative agent, maternal antibodies, or both. Systematic studies have been undertaken to elucidate the genetic factors which govern resistance and susceptibility to lymphomatosis. By progeny-test selection over a period of eight years, Taylor et al. (1915) observed significant differences in the incidence of the disease between resistant and susceptible lines, but could not attribute them to sex-linked genes or to predominantly egg-borne transmission. The method of selection was believed to create a useful degree of relative resistance to lymphomatosis. On the basis of five years of selective breeding under conditions of rigid quarantine, Waters (1915b) demonstrated definite segregation of genes for resistance and susceptibility to lymphomatosis and emphasized the progressive nature of the process in contrast to previous workers who rarely achieved reduction of the disease below the initial percentage incidence. Differences between two sires became apparent when both were mated to the same dam (Waters, 1915c).

Burmester et al. (1960c), in reporting on many years' study with the highly inbred strains of chickens and RPL-12 and Rous sarcoma virus, stated that the several lines responded very differently to exposure to the viruses and that certain of the inbred lines responded differently to the two viruses. Waters and Burmester (1961) presented evidence that in the inbred RPL chickens, susceptibility to intracerebral inoculation with Rous sarcoma virus is dominant to resistance and is dependent for expression on a single pair of autosomal genes.

Work with open-bred stocks does not suggest a simple genetic relationship between tumor resistance and susceptibility. In testing the resistance and susceptibility by artificial exposure, DeOme (1943) found intraperitoneal injections of lymphomatous nerve tissue to cause a more or less parallel increase of the incidence in both the high and low lines. Hendorf et al. (1917), failed to differentiate between resistant and susceptible lines using intraperitoneal injec-

tion of tumorous materials, but obtained significant differences by exposure in the mouth, eyes, or nostrils. This technique failed to increase the incidence of clinical signs in commercial breeding birds sufficiently to make selection more effective.

On the other hand, Carson (1951) exposed chickens from known resistant and susceptible lines to avian leukoses to various viral, bacterial, and protozoal agents without observing a consistent correlation between the genetic constitution for neoplastic and infectious diseases. On the basis of nine years' data, Waters (1951) reported an over-all increase of mortality from lymphomatosis in lines especially selected for susceptibility to this disease, but did not consider such susceptibility or resistance a fixed genetic character. Mortality from other causes failed to show a corresponding increase by his analysis, contrary to the results of Oakberg and Lucas (1919a, b, 1950) on essentially the same material.

Dr. F. B. Hutt established a long-term leukosis project in 1935 which included the development of resistant and susceptible lines. In papers published in 1954 and 1955, Hutt and Cole pointed out the difficulties of providing a standard exposure year after year, particularly of maintaining susceptible populations, and suggested exposing only portions of families in order to have samples of the most susceptible families available for breeding purposes.

In a summary of their work presented by Cole (1962) at the Avian Leukosis Conference, it was reported that for unknown reasons, the level of mortality had decreased in the susceptible strains. Their results over the years have been consistent with the hypothesis that resistance in the strains depends upon many genes. In 1954, samples of line 15 susceptible chicks from East Lansing Laboratory were hatched and raised with the Cornell resistant and susceptible lines. Mortality in the Cornell susceptible lines was approximately 50 per cent whereas mortality in the RPL susceptible line 15 and Cornell resistant strains was on the order of 1 to 4 per cent.

This is most easily interpretable on the basis that the diseases under study at the two laboratories are different.

To attain resistance in the average breeding flock was considered by Coles (1954) to be a 5- to 6-year program. Waters (1954) concluded that hybridization of inbred lines of chickens with various degrees of resistance failed to decrease the mortality from lymphomatosis. The mode of inheritance of resistance to either neural or visceral lymphomatosis could not be elicited.

Bearse et al. (1963), working with lines selected for susceptibility and resistance for 28 generations, reported that when crosses and back crosses were made between resistant and susceptible lines, the leukosis mortality results of the F-1 crosses were between those of the resistant and susceptible lines, whereas mortality from causes other than leukosis was lower than the corresponding mortality in the resistance lines indicating that heterosis was not affecting leukosis mortality but did lower other mortality.

Breeders of commercial chickens believe they have attained a status in which the incidence of tumors in commercial breeding populations is too low for effective selection for resistance. The application of various techniques to increase incidence has either failed to increase the number of tumor cases or has not differentiated between susceptible and resistant families. Unless new means of measuring resistance in breeders can be found, genetic selection cannot be expected to eliminate the problem of leukosis losses.

That sex hormone balance may influence the incidence of lymphomatosis and perhaps activate a latent agent, was suggested by Marine and Rosen (1941). Oakley (1935) already was impressed by the higher incidence of the visceral form in females than in males, while for the osteopetrotic form the reverse seemed to be true (Pugh, 1927; Brandly et al., 1941). Burmester (1945) observed the incidence of natural lymphomatosis to be twice as high in females as in corresponding males during the first 300 days. In further studies along this line, Burmester and Nelson (1945) tested the influence of castration and of sex hormones and credited the male hormone with conferring increased resistance to lymphomatosis, while Davis et al. (1950) believed such altered susceptibility to be a matter of nonspecific hormonal imbalance.

A transmissible agent of fowl leukosis capable of passing through bacteria-retaining filters was first demonstrated by Ellermann and Bang (1908), and subsequently confirmed by many investigators (Jármai, 1930; Furth, 1931a, 1936b; Thomsen and Engelbreth-Holm, 1932; Oberling and Guérin, 1934). A similar etiologic agent was postulated for neurolymphomatosis by Van der Walle and Winkler-Junius (1924), Pappenheimer et al. (1926), Johnson (1932), and others, and for lymphomatosis by Furth (1933). These agents are similar to that of the Rous (Claude and Murphy, 1933; Foulds, 1934) sarcoma in being ultramicroscopic in nature and capable of producing a variety of neoplastic diseases in the fowl (Furth, 1932a), but differ in their inability to cause tumors at the site of injection (Burmester, 1950). They have been termed microplasms, transmissible agents, enzymes (Jármai, 1935), or oncogenic viruses. Oberling and Guérin (1954) could see no valid reason to set such agents apart from ordinary viruses.

The recent advances in the characterization of the avian leukosis viruses which have been sufficiently studied, and the available methodology, have been summarized by Beard (1957a, b).

According to him, the virus of erythroblastosis, if injected intravenously into inbred susceptible chicks, produces recognizable hematologic changes within 2 days and death on the average within 9 days. It lacks the enzymatic capacity to dephosphorylate adenosine triphosphate, and has no Forssman antigen. It is only slightly precipitable by homologous antiserum; whether this is due to the usual, relatively

low concentration of virus particles awaits further study.

By contrast the *virus of myeloblastosis* produces recognizable hematologic changes within 9 days and death on the average in 17 days. It has pronounced capacity to dephosphorylate adenosine triphosphate and a strong Forssman antigen. It is readily precipitable by homologous antiserum, and is usually present in high concentration.

According to Eckert *et al.* (1963) the myeloblastosis virus resembles in size and chemical composition the myxoviruses. The shape of the virus particle is generally believed to be spheroid, but there is a certain percentage of phagelike tailed particles, according to Bartl *et al.* (1963).

The characterization of the *virus of lymphomatosis* is less complete. Burmester and Gentry (1956) observed that RPL-12 filtrates, originally obtained from the Olson (1941) tumor caused "intravascular" lymphomatosis (morphologically indistinguishable from erythroblastosis), with an incubation period of less than 4 months, if given in high doses; and "extravascular" lymphomatosis, with an incubation period up to 9 months, if given in low doses. Both pathologic conditions were accompanied by the occasional occurrence of osteopetrosis, and rare fibrosarcomas and endotheliomas (Burmester, (1957a). In further studies (1957b) the properties of RPL-12 proved to be similar to those of lymphomatosis agents obtained from embryonated eggs, incubator debris, and oral, tracheal, and fecal washings. The RPL-12 filtrates could be neutralized not only by homologous antiserum, but also by that of known erythro- and myeloblastosis, Rous sarcoma, and spontaneous visceral lymphomatosis cases. The question of the etiologic unity of the three main pathologic entities, namely erythroblastosis, myeloblastosis, and osteopetrosis, was left undecided. The positive adenosine triphosphate activity of spontaneous lymphomatosis, reported by Lesher and Burmester (1955), would be at variance with that of known erythro-

blastosis (Bonar *et al.*, 1957). Furthermore, on the basis of cytologic studies, Darcel and Negroni (quoted by Chubb and Gordon, 1957) are inclined to view the conditions produced by RPL-12 as aleukemic variants of erythroblastosis.

In more recent work, Burmester *et al.* (1959b) reported that erythroblastosis and myeloblastosis viruses administered in doses allowing survival of the immediate effects of these diseases produced lymphomatosis and other tumors including osteopetrosis and renal tumors. This paper has not resolved the question of the relationship between tumor viruses, since mixtures of viruses could have occurred.

In a series of papers, the East Lansing workers (Gross *et al.*, 1959; Burmester *et al.*, 1959a, 1960a) describe the pathogenicity of RPL-12 virus and its relationship to the host. The lesions produced are erythroblastosis, visceral lymphomatosis, osteopetrosis, and hemangiomatosis. The level of infective virus applied had a marked effect on the type of neoplasm which developed, with erythroblastosis predominating at high levels of virus and lymphomatosis at low levels. In relationship to the age of the host, sensitivity to intravenous inoculations started decreasing when chicks were about 3 weeks of age and continued to decrease to about 12 weeks. By the oral route, sensitivity decreased very sharply during the first 3 weeks and decreased more slowly beyond that age.

The use of Rous sarcoma virus in tumor investigations has been increasing. Burmester *et al.* (1960b) reported the first recorded transmission of Rous sarcoma under conditions of direct bird-to-bird contact which resulted in the development of Rous sarcomas in the contact birds. In further work, Burmester and Walter (1961) reported the occurrence of visceral lymphomatosis in chickens inoculated with dilute preparations of Rous sarcoma virus which survived to the age at which visceral leukosis occurs.

The complicated serologic interrelationships — based on virus neutralization tests

in vivo — of the three recognized avian leukoses and Fujinami sarcomas, to Rous sarcoma, were summarized and diagrammed by Beard (1957a). The relationships varied from complete mirror neutralization between myeloblastosis and Rous sarcoma to partial or unilateral reactions. Each virus was shown to have a species-specific and a host-specific antigenic component. Beard (1957a) believed the three leukosis agents to constitute biologic entities of a large, closely related family of viruses which may also be responsible for virus-induced avian sarcomas. He speculated that these entities could be mutants of a stem virus, with contagious lymphomatosis providing the mode for perpetuation in nature.

From the beginning, leukosis investigations have been handicapped by a lack of suitable means for detection of the causative agents. Kissling (1947) described a slide leukoagglutination test with formalinized lymphocytes from chickens or various mammals (except oxen) for the diagnosis of avian lymphomatosis and believed the test to be primarily tissue- and not tumor-specific. Darcel (1950) found good correlation of this test with severely affected birds, only slight correlation with clinically "normal" birds, but inconsistencies in consecutive examinations. The potentialities of the phosphatase activity test (Lesher and Burmester, 1955; Anonymous, 1955) have not been explored sufficiently for practical purposes. A report of the United States Bureau of Animal Industry Regional Poultry Research Laboratory (1951) concluded that the only known method for demonstrating the lymphomatosis agent was the inoculation of susceptible chickens.

Kenzy (1953) described the technique for detecting antibodies to Rous sarcoma. The technique utilized *in vitro* incubation of unknown sera with known quantities of Rous virus and subsequent inoculation into chicks. The test was interpreted on the basis of the number and virulence of tumors developing in the inoculated

chicks. A subsequent paper (Kenzy and Neuzil, 1953) reported high percentages of sera containing Rous neutralizing antibodies from flocks with high losses from leukosis and lower levels of Rous neutralizing antibodies in birds which were grossly and microscopically normal.

Rubin, who had developed a tissue culture assay technique for Rous sarcoma virus, reported (1960) the presence of a virus in chicken embryos which induced resistance in tissue culture to superinfection with Rous sarcoma virus. The virus was shown to be associated with leukosis and related antigenically to RPL-12. The technique was developed into a test for the virus and it was shown that antibody to the resistance-inducing factor (RIF) was very closely related to Rous virus antigenically so that direct Rous neutralization tests were an excellent measure of the leukosis virus antibody. Epidemiologic studies (Rubin et al., 1961) using these tests showed that congenital transmission of the virus was very frequent in the population under study, that passive immunity was effective in delaying contact infection until chicks were about 6 weeks of age, and that spread of the virus continued until all birds in contact were infected.

Rubin has continued his studies with Rous sarcoma virus. Of possible significance in the study of leukosis is his detection of a second virus in standard stocks of Rous sarcoma (Rubin and Vogt, 1962) and the later demonstration by Hanafusa et al. (1963) that Rous sarcoma is defective and requires a helper virus, presumably one of the resistance-inducing group, to complete its biologic cycle.

Sevoian et al. in a series of papers (1962, 1963a, and 1963b) described an agent causing neural and visceral lymphomatosis. This virus, labeled JM, produces lesions in a relatively short time after infection, 2 to 5 weeks, in the nerves and particularly the gonads as well as other visceral organs. Neural lesions tend to predominate in young birds and visceral lesions in older birds, duplicating in part field cases of

lymphomatosis. The agent was shown to be transmitted via the air-borne route. The only test for the virus currently available is the inoculation of susceptible chicks.

Present evidence suggests that JM virus and the resistance-inducing leukosis viruses are unrelated. Calnek (1963) has stated that he believes the JM virus does not induce resistance to Rous virus. Burmester has provided evidence that RPL-12 is not spread by the air-borne route and observation resulting from Rubin's studies would support this observation, whereas the JM virus has been demonstrated to be infectious by the air-borne route. Confirmatory evidence must await new means of assay of the JM virus.

Most European investigators have separated the leukosis complex into lymphoid, myeloid and erythroleukosis, and Marek's disease. Campbell (1961) has proposed a classification system for these diseases. Biggs (1961) concluded that the cells from Marek's disease could not be differentiated histologically from those of lymphoid leukosis on histopathologic grounds alone. He reported that there may be a difference in the competence of cells in cell suspensions, with those from Marek's disease retaining their competence and those from RPL-12 lymphoid tumors showing no competence in the graft against host reaction in chicken embryos.

The status of information about Marek's disease does not allow any determination as to its relation with JM virus, though it seems likely that it can be differentiated from the RPL-12 type viruses.

The Rous resistance-inducing test provides the first useful *in vitro* test for the detection of a leukosis virus and its antibody. Of foremost interest are tests to detect viruses of the Marek and JM types and a simpler test for resistance-inducing viruses.

TRANSMISSION

The investigations of Burmester and the Regional Poultry Laboratory staff and of Rubin *et al.* (1961) have very definitely es-

tablished the mode of transmission of the resistance-inducing leukosis viruses. The literature, as indicated in the following discussion, points up the serious difficulty investigators through the years have encountered in studying leukosis. One of the most confusing problems in determining the method of transmission of infection has been that the gross manifestation of disease (the tumor) is not an invariable consequence of infection with the virus.

The earlier contentions of Doyle (1928) and McGaughey and Downie (1930) on the transmission of fowl paralysis through the egg did not find support in direct embryo inoculation experiments with this disease by McLennan (1935) or with erythroleukosis by Jármai (1933). Gibbs and Johnson (1955) claimed to have observed the characteristic pathologic cells of neurolymphomatosis in the follicular or seminal fluid of affected birds, while Storti and Mezzadra (1938) observed survival but not multiplication of the leukosis virus in 4-day-old chicken embryos. Van den Berghe and d'Ursel (1939) apparently transmitted the leukosis agent to chicken embryos, and Pollard and Hall (1941) to embryos of other avian species. Extensive breeding experiments by Lee and Wilcke (1941) indicated that the incidence of the leukosis complex is much higher in the progeny from iritis-affected birds than from normal birds, especially if either the female or both parents were affected. Carrying on a similar breeding experiment, Durant and McDougle (1939) and Lee and Wilcke (1941) were able to demonstrate the agent of fowl paralysis in the blood of recently hatched normal chicks. A well-planned, practical transmission experiment with neural lymphomatosis by Gordon *et al.* (1955) suggested spread by contact, but the possibility of transmission via the egg could not be ruled out.

Waters (1945a) pointed out the importance of the egg as a carrier, since simple importation of hatching eggs into an entirely new isolated poultry plant resulted in the spontaneous appearance of

lymphomatosis in chickens within 40 days after hatching. Later, Cottral et al. (1949) actually accomplished the transmission of visceral lymphomatosis with tissues from 15- and 18-day-old embryos and newly hatched chicks, derived from parents free from gross evidence of the disease up to the age of 600 days.

On the basis of these inductive experiments, avian lymphomatosis was ranged among the egg-borne carrier diseases by Cottral (1949).

In contrast to this view, Hutt and Cole (1947) questioned the importance of egg transmission of lymphomatosis, especially in comparison with genetic and environmental factors, and also referred to the work of Carr (1945), who failed to observe egg transmission in Rous sarcoma. Cole (1949) summarized the evidence pro and con for egg transmission and minimized its importance. Analyzing the results with resistant and susceptible birds kept under controlled environmental conditions, Cole and Hutt (1951) presented evidence that leukosis is not transmitted through the eggs. The discrepancy may be explained, in part, by the fact that Cole and Hutt were dealing primarily with neural lymphomatosis for which egg transmission has not been demonstrated.

On the basis of field observations, pathologists with large commercial breeders of egg-laying chickens who have recorded major leukosis losses, agree that there is no direct relationship between egg or hatchery transmission of virus and severe leukosis losses in commercial poultry flocks. This conclusion has been reached on the basis of observations of large numbers of shipments of chicks over a period of many years.

The question of transmission of lymphomatosis by contact has been subjected anew to experimental inquiry. Barber (1942, 1913) reported a lower incidence of leukotic diseases in comparable groups of birds reared away from, than on, known infected premises. In analyzing neoplastic mortality in sexually mature birds over a period of seven years, Hutt et al. (1944)

observed a decreased incidence, independent from genetic background, in lots of birds that had been brooded for the first two weeks about 200 feet (in comparison with 40 feet for the controls) away from old birds. Environmental factors other than proximity to infected adults, such as restriction of food intake, crowding, and rapid growth-inducing rations, increased the mortality during the first six weeks, but failed to enhance the incidence of leukotic diseases during the succeeding 65 weeks (Cole and Hutt, 1949). Studies on the influence of the environment culminated in the development, by a system of selective breeding and extremely rigid sanitation, of a strain of birds essentially free from lymphomatosis by Waters and Prickett (1944). The unique contagious nature of lymphomatosis in chickens, in contrast to most mammalian and avian tumors, was brought out by Waters (1947). Support for this thesis has come from the work of Brewer and Brownstein (1947) in this country and Harriss et al. (1947) in England.

Waters and Bywaters (1919) made the observation that contact transmission, probably via the respiratory tract, may already take place in the incubator. Continuing this line of thought, the workers at the Regional Laboratory were able to show the presence of the lymphomatosis virus in incubator debris and in most of the secretions and excretions. In this connection, two new observations contributed materially to the present-day concept of spread of lymphomatosis: the shedding of the virus not only by affected chickens but also by apparently normal chickens (Burmester, 1956); and the demonstration of passive antibodies in chicks from hyperimmunized dams (Burmester et al., 1957). Although such antibodies may be protective, they are not sufficiently strong to neutralize the virus which may coexist in their presence.

The recent epidemiologic studies of Rubin et al. (1962) with the resistance-inducing test have confirmed and extended these observations. Using this test Hughes

lymphomatosis. The agent was shown to be transmitted via the air-borne route. The only test for the virus currently available is the inoculation of susceptible chicks.

Present evidence suggests that JM virus and the resistance-inducing leukosis viruses are unrelated. Calnek (1963) has stated that he believes the JM virus does not induce resistance to Rous virus. Burmester has provided evidence that RPL-12 is not spread by the air-borne route and observation resulting from Rubin's studies would support this observation, whereas the JM virus has been demonstrated to be infectious by the air-borne route. Confirmatory evidence must await new means of assay of the JM virus.

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TRANSMISSION

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be remembered that temporary remissions of the clinical signs may occur spontaneously.

With the new recognition of the role of the bursa of Fabricius in leukemogenesis, reduction of its influence on the incidence of lymphomatosis has been approached experimentally in two ways: dipping of eggs in testosterone propionate by Glick (1963), and bursectomy before the age of one month by Peterson et al. (1963).

In vaccination attempts for the prevention of the avian leukosis complex, Fritzsche (1938) failed to immunize birds against fowl paralysis with formol-treated tissue vaccine. Uhl (1938) secured some degree of immunity against erythrogranuloblastosis with aluminum hydroxide-adsorbed tissues. Johnson (1945) tried out several types of vaccines with inconclusive results. The demonstrated oncolytic action of certain neurotropic viruses on avian lymphoid tumors is of interest (Sharpless et al., 1950). The first tangible results were obtained by Burmester et al. (1957) who found that various types of vaccines, except heat-killed ones, repeatedly administered to chickens under one year of age, conferred a substantial immunity to their progeny. This work was of major consequence because it established the fact that the well-known principle of passive immunity held for visceral lymphomatosis. A concomitant reduction in the incidence of osteopetrosis was of taxonomic interest. At present the practical expectations from this work should be tempered.

Although an absolute control program for the avian leukosis complex cannot be suggested, there are two major avenues of approach, namely by breeding for resistance and by sanitation. In view of the pathologic and apparent etiologic diversity of the disease group, the effectiveness of a control program will vary with the prevalence of the respective forms. Thus, of the most common ones, neural lymphomatosis seems to respond more to selection, visceral lymphomatosis to sanitation. That there are puzzling failures is indicated by the reported increased incidence of visceral lymphomatosis even in broiler flocks (Benton and Cover, 1957; Benton et al., 1962), and turkeys (Simpson et al., 1957).

While the interaction of heredity and infectious agents in the environment, starting with the formation of the egg, is not fully understood, a disease complex estimated to cause losses in the United States of $1,000,000 per week (Hutt, 1944) calls for the intelligent application of all available facts.

In general, birds showing clinical evidence of the avian leukosis complex, including true ocular lymphomatosis, should be consistently culled. Frequent laboratory checkups on the causes of mortality are advisable.

1. By systematic selection of the progeny for resistance to lymphomatosis, it is possible to obtain relatively resistant strains of birds (Taylor et al., 1943). In this it is preferable to choose breeders whose brothers and sisters have shown the lowest incidence of the avian leukosis complex (Gildow et al., 1940). Insofar as is known, the progeny of resistant stock remains fully susceptible (Blakemore, 1959). A selective breeding program for general livability also tends to decrease the incidence of avian leukotic diseases, according to Bryant and Johnson (1944). Under practical conditions, breeding from the survivors of natural selection and especially maintenance of a "closed flock" with a minimum of importations have given the most tangible results.

Although ordinary sanitary methods are generally conceded to be insufficient to prevent entirely the occurrence of avian lymphomatosis (Waters, 1945a; Harriss et al., 1947), hygiene as a factor in the control of the avian leukosis complex has been shown to be of major importance. On the thesis that control of the avian leukosis complex should be achieved by rearing genetically resistant stock in the best possible environment, Hutt (1951) and Hutt and Cole (1954) have clearly set forth a program which combines the genetic and sanitary features and is adaptable to various managerial situations.

et al. (1963), succeeded in producing an experimental flock of chickens free of the resistance-inducing viruses. Levine and Nelsen (1964) followed a similar approach but found a significant portion of the breeding hens (25 per cent) to lack antibodies to Rous virus and RIF agents in their progeny, perhaps indicating no infection in a known infected environment. It seems that individual criteria have to be established in attempts to develop RIF-free flocks. Furthermore the demonstration of occasional genetic resistance of chicken embryos to Rous virus by Crittenden et al. (1963) and the report of antigenic plurality of Rous virus by Simons and Dougherty (1963) and its confirmation by Bang and Foard (1963) may complicate the interpretation of the RIF test.

The available information on the natural and artificial transmission of avian lymphomatosis has been reviewed by Burmester (1957a) and Gross (1957). Visceral lymphomatosis was considered to be the only known contagious avian tumor. The resistance-inducing viruses may be shed in nearly all eggs laid by tolerant hens and in occasional eggs laid by hens with circulating antibody (Rubin et al., 1962). This virus spreads to pen mates by intimate contact. Available evidence suggests that spread is very limited by indirect means.

The JM virus described by Sevoian and Chamberlain (1963) appears to be an unrelated virus. Early evidence suggests that it is spread via the air-borne route as well as by contact. Although no definite study has yet been reported, if the JM virus is related to the condition observed by Hutt and Cole over many years or to the agent causing neural losses in the field, egg transmission is not likely to be an important factor in the development of such tumors.

The presence of the avian leukoses agents in the blood stimulated research on blood sucking parasites as intermediaries. While most of the experiments were negative (Olson, 1940), Johnson (1937) showed the common red mite, Dermanys-

sus gallinae, and Brown and Cross (1941) the Texas "blue bug", Argas persicus, to be possible mechanical vectors of the lymphomatosis agent. Although not supported by experimental evidence, Carr (1952) re-emphasized the importance of blood-sucking parasites in the transmission of the diseases of the avian leukosis complex. This opinion was recently supported by successful laboratory transmission of Rous sarcoma virus by Aedes aegypti and A. albopictus (McDaniel et al., 1962). In further studies the same authors (1964) found the virus to survive and perhaps multiply in Culex pipiens pipiens, which commonly attacks birds, for at least 16 days. Johnson (1937) also suggested that minor operations, as in fowl pox vaccination, may have the same effect.

On the belief that the egg may be invaded by the lymphomatosis agent both at the time of formation and after commencing its independent existence, Cottral (1949) pointed to the danger of incorporating this agent in live virus vaccines, made from so-called "normal" embryos. Support for this view has come from the experience of Piedrafita (1951), who observed severe outbreaks of avian leukosis following the use of a formol-killed Newcastle vaccine.

TREATMENT AND CONTROL

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be remembered that temporary remissions of the clinical signs may occur spontaneously.

With the new recognition of the role of the bursa of Fabricius in leukemogenesis, reduction of its influence on the incidence of lymphomatosis has been approached experimentally in two ways: dipping of eggs in testosterone propionate by Glick (1963), and bursectomy before the age of one month by Peterson et al. (1963).

In vaccination attempts for the prevention of the avian leukosis complex, Fritzsche (1938) failed to immunize birds against fowl paralysis with formol-treated tissue vaccine. Uhl (1938) secured some degree of immunity against erythrogranuloblastosis with aluminum hydroxide-adsorbed tissues. Johnson (1945) tried out several types of vaccines with inconclusive results. The demonstrated oncolytic action of certain neurotropic viruses on avian lymphoid tumors is of interest (Sharpless et al., 1950). The first tangible results were obtained by Burmester et al. (1957) who found that various types of vaccines, except heat-killed ones, repeatedly administered to chickens under one year of age, conferred a substantial immunity to their progeny. This work was of major consequence because it established the fact that the well-known principle of passive immunity held for visceral lymphomatosis. A concomitant reduction in the incidence of osteopetrosis was of taxonomic interest. At present the practical expectations from this work should be tempered.

Although an absolute control program for the avian leukosis complex cannot be suggested, there are two major avenues of approach, namely by breeding for resistance and by sanitation. In view of the pathologic and apparent etiologic diversity of the disease group, the effectiveness of a control program will vary with the prevalence of the respective forms. Thus, of the most common ones, neural lymphomatosis seems to respond more to selection, visceral lymphomatosis to sanitation. That there are puzzling failures is indicated by the reported increased incidence of vis-

ceral lymphomatosis even in broiler flocks (Benton and Cover, 1957; Benton et al., 1962), and turkeys (Simpson et al., 1957).

While the interaction of heredity and infectious agents in the environment, starting with the formation of the egg, is not fully understood, a disease complex estimated to cause losses in the United States of $1,000,000 per week (Hutt, 1944) calls for the intelligent application of all available facts.

In general, birds showing clinical evidence of the avian leukosis complex, including true ocular lymphomatosis, should be consistently culled. Frequent laboratory checkups on the causes of mortality are advisable.

1. By systematic selection of the progeny for resistance to lymphomatosis, it is possible to obtain relatively resistant strains of birds (Taylor et al., 1943). In this it is preferable to choose breeders whose brothers and sisters have shown the lowest incidence of the avian leukosis complex (Gildow et al., 1940). Insofar as is known, the progeny of resistant stock remains fully susceptible (Blakemore, 1939). A selective breeding program for general livability also tends to decrease the incidence of avian leukotic diseases, according to Bryant and Johnson (1944). Under practical conditions, breeding from the survivors of natural selection and especially maintenance of a "closed flock" with a minimum of importations have given the most tangible results.

Although ordinary sanitary methods are generally conceded to be insufficient to prevent entirely the occurrence of avian lymphomatosis (Waters, 1945a; Harriss et al., 1947), hygiene as a factor in the control of the avian leukosis complex has been shown to be of major importance. On the thesis that control of the avian leukosis complex should be achieved by rearing genetically resistant stock in the best possible environment, Hutt (1951) and Hutt and Cole (1954) have clearly set forth a program which combines the genetic and sanitary features and is adaptable to various managerial situations.

et al. (1963), succeeded in producing an experimental flock of chickens free of the resistance-inducing viruses. Levine and Nelsen (1964) followed a similar approach but found a significant portion of the breeding hens (25 per cent) to lack antibodies to Rous virus and RIF agents in their progeny, perhaps indicating no infection in a known infected environment. It seems that individual criteria have to be established in attempts to develop RIF-free flocks. Furthermore the demonstration of occasional genetic resistance of chicken embryos to Rous virus by Crittenden *et al.* (1963) and the report of antigenic plurality of Rous virus by Simons and Dougherty (1963) and its confirmation by Bang and Foard (1963) may complicate the interpretation of the RIF test.

The available information on the natural and artificial transmission of avian lymphomatosis has been reviewed by Burmester (1957a) and Cross (1957). Visceral lymphomatosis was considered to be the only known contagious avian tumor. The resistance-inducing viruses may be shed in nearly all eggs laid by tolerant hens and in occasional eggs laid by hens with circulating antibody (Rubin *et al.*, 1962). This virus spreads to pen mates by intimate contact. Available evidence suggests that spread is very limited by indirect means.

The JM virus described by Sevoian and Chamberlain (1963) appears to be an unrelated virus. Early evidence suggests that it is spread via the air-borne route as well as by contact. Although no definite study has yet been reported, if the JM virus is related to the condition observed by Hutt and Cole over many years or to the agent causing neural losses in the field, egg transmission is not likely to be an important factor in the development of such tumors.

The presence of the avian leukoses agents in the blood stimulated research on blood sucking parasites as intermediaries. While most of the experiments were negative (Olson, 1940), Johnson (1937) showed the common red mite, *Dermanys-*

sus gallinae, and Brown and Cross (1941) the Texas "blue bug," *Argas persicus,* to be possible mechanical vectors of the lymphomatosis agent. Although not supported by experimental evidence, Carr (1952) re-emphasized the importance of blood-sucking parasites in the transmission of the diseases of the avian leukosis complex. This opinion was recently supported by successful laboratory transmission of Rous sarcoma virus by *Aedes aegypti* and *A. albopictus* (McDaniel *et al.*, 1962). In further studies the same authors (1964) found the virus to survive and perhaps multiply in *Culex pipiens pipiens,* which commonly attacks birds, for at least 16 days. Johnson (1937) also suggested that minor operations, as in fowl pox vaccination, may have the same effect.

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——: 1936. Primary irido-cyclitis in fowls: a condition distinct from the eye lesions occurring in neuro-lymphomatosis. Jour. Comp. Path. and Therap. 49:310.

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——: 1957b. Physical methods for the analysis of cells. Ann. N.Y. Acad. Sci. 69 (Art. 4):530.

——: 1963a. Avian virus growths and their etiologic agents. Adv. in Cancer Res. 7:1.

——: 1963b. Viral tumors of chickens with particular reference to the leukosis complex. Ann. N.Y. Acad. Sci. 108:1057.

Bearse, G. E., Becker, W. A., and Hamilton, C. M.: 1963. Resistance and susceptibility to the avian leukosis complex in chickens. Poultry Sci. 42:110.

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——, and March, B. E.: 1959. Genetic and nutritional effects on the incidence of the avian leukosis complex. Poultry Sci. 38:1103.

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——: 1961. Some observations on the properties of cells from the lesions of Marek's disease and lymphoid leukosis. Proc. 13th Symposium. Colston Res. Society, Butterworth's Scient. Publ., London. Pp. 83.

——: 1963. The avian leukosis complex. Poultry Res. 3.3.

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——: 1939. The nature of fowl paralysis (neurolymphomatosis). Jour. Comp. Path. and Therap. 52:144.

——, and Dalling, T.: 1939. Some recent observations on fowl paralysis (neurolymphomatosis). Proc. Seventh World's Poultry Cong., p. 282.

Blaxland, J. D.: 1956. The practical importance of leukosis and fowl paralysis. Vet. Record 68.528.

Bloom, W., Bloom, M. A., and McLean, F. C.: 1941. Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeons. Anat. Record 81:443.

Blount, W. P.: 1932. Studies of fowl paralysis. III. Gastronomic enteritis. Vet. Jour. 88.236.

——: 1934. Fowl paralysis. Vet. Record 14:469.

——: 1939. Hemocytoblastosis. Vet. Jour. 95:203.

Bonar, R. A., Beaudreau, G. S., Sharp, D. G., Beard, D., and Beard, J. W.: 1957. Virus of avian erythroblastosis. V. Adenosinetriphosphatase activity of blood plasma from chickens with the disease. Jour. Nat. Cancer Inst. 19:909.

——, Heine, U., Beard, D., and Beard, J. W.: 1963. Virus of avian myeloblastosis (BAI strain A). XXIII. Morphology of virus and comparison with strain R (erythroblastosis). Jour. Nat. Cancer Inst. 30.949.

Brandly, C. A.: 1941. Progress report on several phases of pathology research. Rep. Second Collab. Conf. U.S. Reg. Poultry Res. Lab., East Lansing, Mich., 23:38.

——, Nelson, N. M., and Cottral, G. E.: 1941. Serial passage of strain 3. lymphomatosis-osteopetrosis in chickens. Jour. Am. Vet. Med. Assn. 99.219.

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REFERENCES

Adamstone, F B 1936 A lymphoblastoma occurring in young chicks reared on a diet treated with ferric chloride to destroy vitamin E. Am. Jour. Cancer 28 540.

Ahlstrom, C. G., Bergman, S., Forsby, N., and Jonsson, N.. 1963. Rous sarcoma in mammals. Acta Un Int Cancer 19 294

Andersen, C. W., and Bang, O : 1928. La leucémie ou leucose transmissible des poules, Festskrift til Prof. Bernhard Bang. Kandrup and Wunsch, Kopenhavn. P. 353.

Andrews, C. H., and Glover, R E : 1939. A case of neurolymphomatosis in a turkey, Vet. Record 51 934

Anonymous: 1955 Now — a test for lymphomatosis, World's Poultry Sci. Jour. 11·30.

Asmundon, V S., and Biely, J 1932. Inheritance of resistance to fowl paralysis (neurolymphomatous gallinarum). 1. Differences in susceptibility. Canad. Jour. Res. 6:171.

Asplin, F D : 1944. Treatment of a virus disease of chickens with sulfonamides. Nature, London, 153 253

———: 1947a. Observations on the aetiology of lymphomatosis. II. The association of "chick disease" virus with field cases of lymphomatosis. Jour. Comp Path. and Therap. 57:126.

———: 1947b. Observations on the aetiology of lymphomatosis. III. The development of lymphomatosis in chickens free of the "chick disease" virus. Jour. Comp. Path. and Therap. 57:154.

Ball, R. F. 1942. The effect of the ration upon iris color of Single Comb White Leghorns. Poultry Sci. 25 577.

———. 1944. A study of iris depigmentation in Single Comb White Leghorns Doctoral Thesis. Cornell Univ.

———, and Cole, R K : 1946. A study of the relationship between the iris color of the dam and the mortality of her progeny. Poultry Sci. 25:33.

Bang, F. B., and Foard, M : 1963 Flocks of chickens free from antibody to Rous virus. Jour Nat. Cancer Inst 50:457

Barber, C. V: 1942. The effect of the rearing environment upon the incidence of avian leucosis complex. Cornell Vet. 32:194

———: 1943. The effect of environment on the incidence of avian-leukosis complex lesions among resistant and nonresistant chickens. Cornell Vet. 33·78.

Bartl, P, Riman, J, and Sorm, F : 1963. The problem of the phage like structure of the avian leukosis virus. Experientia 19:635

Battaglia, F., and Leinati, L. 1929. Malattie sistemiche transmissibili degli organi emopoietici del pollo con ricerche sugli elementi morfologici del sangue normale e loro genesi. Boll. d. Inst. Sieroterap. Milanese. 8 9·31; 73–94; 183–98.

Bayon, H. P.: 1929. The pathology of transmissible anaemia (erythromyelosis) in the fowl. Parasitology 21:539.

———. 1950. The comparative pathology of anaemia and leucocythemia in fowls. Jour. Comp. Path. and Therap. 43:188.

———. 1951. Acute neuro-lymphomatosis gallinarum in a strain of Rhode Island Red fowls. Vet. Record 11:907.

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———: 1931. Acute *neuro-lymphomatosis gallinarum* in a strain of Rhode Island Red fowls. Vet. Record 11:907.

———: 1932. The pathogenesis of *neurolymphomatosis gallinarum* and similar forms of "fowl paralysis." Vet. Record 12:457.

———: 1936. Primary irido-cyclitis in fowls: a condition distinct from the eye lesions occurring in *neuro-lymphomatosis. Jour. Comp. Path. and Therap.* 49:310.

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———: 1957a. Etiology of avian leukosis. Ann. N.Y. Acad. Sci. 68 (Art. 2):473.

———: 1957b. Physical methods for the analysis of cells. Ann. N.Y. Acad. Sci. 69 (Art. 4):530.

———: 1963a. Avian virus growths and their etiologic agents. *Adv. in Cancer Res.* 7:1.

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———: 1961. Some observations on the properties of cells from the lesions of Marek's disease and lymphoid leukosis. Proc. 13th Symposium. Colston Res. Society, Butterworth's Scient Publ, London. Pp. 83.

———: 1965. The avian leukosis complex. Poultry Rev. 3:3.

———, and Payne, L. N.: 1963. Transmission experiments with Marek's disease (fowl paralysis). Vet. Record 75:177.

Blakemore, F.: 1934. The leucocytes of fowl blood with special reference to fowl paralysis. Vet. Record 14:417.

———: 1939. The nature of fowl paralysis (neurolymphomatosis). Jour. Comp. Path. and Therap. 52:144.

———, and Dalling, T.: 1939. Some recent observations on fowl paralysis (neurolymphomatosis). Proc. Seventh World's Poultry Cong., p. 282.

Blaxland, J. D.: 1956. The practical importance of leucosis and fowl paralysis. Vet. Record 68:528.

Bloom, W., Bloom, M. A., and McLean, F. C.: 1941. Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeons. Anat. Record 81:443.

Bloom, W. P.: 1932. Studies of fowl paralysis. I. Gastronomic enteritis. Vet. Jour. 88 236.

———: 1934. Fowl paralysis. Vet. Record 14:469.

———: 1959. Hemocytoblastosis. Vet. Jour. 95.91.

Bonar, B. A., Beaudreau, G. S., Sharp, D. G., Beard, D., and Beard, J. W.: 1957. Virus of avian erythroblastosis. V. Adenosinetriphosphatase activity of blood plasma from chickens with the disease. Jour. Nat. Cancer Inst. 19.909.

———, Heine, U., Beard, D., and Beard, J. W.: 1963. Virus of avian myeloblastosis (BAI strain A). XXIII. Morphology of virus and comparison with strain R (erythroblastosis). Jour. Nat. Cancer Inst. 30.949.

Brandly, C. A.: 1941. Progress report on several phases of pathology research. Rep. Second Collab. Conf. U.S. Reg. Poultry Res. Lab., East Lansing, Mich., 29.58.

———, Nelson, N. M., and Cottral, G. E.: 1941. Serial passage of strain 3, lymphomatosis osteopetrosis in chickens. Jour. Am. Vet. Med. Assn 99:219.

———, Thorp, F., and Prickett, G. O.: 1949. Response of chicken embryos to tissues of chickens affected with the avian leukosis complex and to tissues of normal birds. Poultry Sci. 28.486.

Brewer, N. R., and Brownstein, B.: 1946. The transmission of lymphomatosis in the fowl. Am. Jour. Vet. Res. 7:123.

Bridges, C. H., and Flowers, A. I.: 1958. Iridocyclitis and cataracts associated with an encephalomyelitis in chickens. Jour. Am. Vet. Med. Assn. 132:79.

Brion, A., and Fontaine, M.: 1965. Données récentes sur les virus de leucoses aviaires. Revue de Pathologie Générale 63:317.

Brochet, L.: 1935. Ostéite hypertrophiante chez la poule. Bul. Acad. vét. Fr. 88:191-96 and 477.

Brown, J. C., and Cross, J. C: 1941. A probable agent for the transmission of fowl paralysis. Science 93.528.

Bryant, R. L., and Johnson, E. P.: 1944. Incidence of mortality in two strains of Single Comb White Leghorn chickens. Poultry Sci. 23.521.

Bullis, K. L., Snoeyenbos, G. H., and Van Rockel, H.: 1950. A keratoconjunctivitis in chickens. Poultry Sci. 29:386.

Burmester, B. R.: 1945. The incidence of lymphomatosis among male and female chickens. Poultry Sci. 24:469.

——— : 1947a. Centrifugation of a filtrable agent inducing osteopetrosis and lymphoid tumors in the domestic fowl. Poultry Sci. 26 215.

——— : 1947b. Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell-free preparations. Cancer Res. 7:786.

——— : 1947c. Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell-free preparations. (Absl.) Poultry Sci. 26-534.

——— : 1947d. The cytotoxic effect of an avian lymphoid tumor antiserum. Cancer Res. 7:459.

——— : 1950. The effect of storage at low temperature on the viability of several avian lymphoid tumor strains. Cancer Res. 10:708.

——— : 1952. Studies on fowl lymphomatosis. Ann. N.Y. Acad. Sci. 54(Art. 6):992.

——— : 1956 The shedding of the virus of visceral lymphomatosis in the saliva and feces of individual normal and lymphomatous chickens. Poultry Sci. 35:1089.

——— : 1957a. Routes of natural infection in avian lymphomatosis. Ann. N.Y. Acad. Sci. 68(Art. 2):487

——— : 1957b. Pathology report for September. Private communication

——— : 1962 The oncogenic spectrum of the fowl tumor virus strains and some influencing factors. Avian Leukosis Conference, U.S.D.A., Regional Poultry Res. Lab., East Lansing, Mich. P. 18.

———, and Belding, T. C. 1947. Immunity and cross immunity reactions obtained with several avian lymphoid tumor strains. Amer. Jour. Vet. Res. 8:128.

———, Brandly, C. A., and Prickett, C. O.. 1944. Viability of a transmissible fowl tumor (Olson) upon storage at low temperatures. Proc. Soc. Exper. Biol. and Med. 55:205.

———, and Cottral, G. E.: 1947. The propagation of filtrable agents producing lymphoid tumors and osteopetrosis by serial passage in chickens. Cancer Res. 7:669.

———, and Denington, E. M: 1947. Studies on the transmission of avian visceral lymphomatosis. I. Variation in transmissibility of naturally occurring cases. Cancer Res. 7:779.

———, Fontes, A. K., and Walter, W. G.: 1960a. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. III. Influence of host age and route of inoculation. Jour. Nat. Cancer Inst. 24.1423.

———, Fontes, A. K., and Walter, W. G.: 1960b. Contact transmission of Rous sarcoma. Jour. Nat. Cancer Inst. 25 307.

———, Gross, M. A., Walter, W. G., Bryan, W. R., and Groupé, V: 1960c. The response of several inbred lines of White Leghorns to inoculation with the viruses of strain RPL-12 visceral lymphomatosis-erythroblastosis and of Rous sarcoma. Poultry Sci. 39:199.

———, and Fredrickson, T. N: 1964. Transmission of virus from field cases of avian lymphomatosis. I. Isolation of virus in line 15 I chickens. Jour. Nat. Cancer Inst. 32:37.

———, and Gentry, R. F. 1956. The response of susceptible chickens to graded doses of the virus of visceral lymphomatosis Poultry Sci. 35:17.

———, Gross, M. A., Walter, W. G., and Fontes, A. K 1959a Pathogenicity—II. Host virus interrelations affecting response. Jour. Nat Cancer Inst. 22.103.

———, Lucas, A. M., Gross, M. A., Walter, W. G., Darcel, C. le Q, Defendi, V., Jones, O. P., Jungherr, E., Olson, C., and McKee, G. S.: 1959c. Conference on histopathology of experimental avian lymphomatosis Am Jour. Vet. Res. 20 223.

———, and Nelson, N. M. 1945 The effect of estrogen and sex hormones upon the incidence of lymphomatosis in chickens. Poultry Sci. 24:509.

———, and Prickett, C O: 1944 Immunity reactions obtained with a transmissible fowl tumor (Olson). Cancer Res. 4:364.

———, and Prickett, C O: 1945. The development of highly malignant tumor strains from naturally occurring avian lymphomatosis. Cancer Res. 5:652.

Prickett, G. O., and Belding, T. C. 1946a. A filtrable agent producing lymphoid tumors and osteopetrosis in chickens. Cancer Res. 6:189.

———, Prickett, C. O. and Belding, T. C: 1946b. The occurrence of neural and visceral lymphomatosis in chickens proven immune to transplants of lymphoid tumor strains. Poultry Sci. 6:616.

———, Sharpless, G. R., and Fontes, A K.: 1960d. Virus isolated from avian lymphomas unrelated to lymphomatosis virus. Jour. Nat. Cancer Inst 24:1443

———, and Walter, W. G.: 1961. Occurrence of visceral lymphomatosis in chickens inoculated with Rous sarcoma virus. Jour. Nat. Cancer Inst. 26:511.

———, Walter, W. G., and Fontes, A. K.: 1957. The immunological response of chickens after treatment with several vaccines of visceral lymphomatosis. Poultry Sci. 36:79.

———, Walter, W. G., Gross, M. A., and Fontes, A. K.: 1959b The oncogenic spectrum of two "pure" strains of avian leukosis. Jour. Nat. Cancer Inst. 23:277.

Butler, W. J., and Warren, D. M.: 1938. Fowl leukemia and vitamin E. Jour. Am. Vet. Med Assn. 92:204.

———, Warren, D. M., and Hammersland, H. L.: 1938. Nutrition as a factor in the incidence of fowl leukosis. Jour. Am. Vet. Med. Assn. 93 307.

Calneck, B. J.: 1963. Personal communication from Cornell Vet. College.

Campbell, J. G.: 1954. Avian leucosis: A plea for clarification. Proc. 10th World's Poultry Cong. Edinburgh, Scotland. P. 193.

———: 1956. Leucosis and fowl paralysis compared and contrasted. Vet. Record 68:527.

———: 1961. A proposed classification of the leukosis complex and fowl paralysis. British Vet. Jour. 117:316.

———: 1963. Virus-induced tumours in fowls. The clinical and pathological characteristics of the avian leucosis complex, with special reference to Rous and allied tumours. Proc. Roy Soc. Med. 56:305.

Caranti, V.: 1956. Note ed osservazioni personali sui rapporti tra paralisi di Marek e coccidiosi. Profilassi. 29:133.

Carr, J. G.: 1945. Lack of transmission of avian tumour virus from carrier hens to their offspring via the egg. Proc. Roy. Soc., Edinburgh, Ser. B, 62.54

———: 1952. The leucosis complex. World's Poultry Sci. Jour. 8:276.

———: 1956. Renal adenocarcinoma induced by fowl leukaemia virus. British Jour. Cancer 10:379.

———: 1959. A survey of fowl tumors for induction of kidney carcinomas. Virology 8.269.

———: 1962. Observations on the haemorrhagic disease induced by fowl tumour viruses. British Jour. Cancer 16:626.

Carson, J. R.: 1951. Exposure to disease agents of strains of chickens differing in resistance to leucosis. Poultry Sci. 30:213.

Chubb, L. G., and Gordon, R. F.: 1957. The avian leucosis complex—a review. Vet. Rev. and Annotations 3 (Part 2):97.

———, and Laursen, A. L.: 1954. Further observations on the effect of aminopterin. A methopterin and citrovorum factor on the growth of transplantable avian lymphoid tumours. Brit. Jour. Pharmacol. and Chemotherapy 9:419.

Claude, A., and Murphy, J. B.: 1933. Transmissible tumors of the fowl. Physiol. Rev. 13:246.

Cole, R. K.: 1941. Genetic resistance to a transmissible sarcoma in the fowl. Cancer Res. 1:714.

———: 1949. The egg and avian leukosis. Poultry Sci. 28:31.

———: 1962. Cornell's experience in breeding for resistance to leukosis, Avian Leukosis Conference, U.S.D.A. Regional Poultry Res. Lab., East Lansing, Mich. P. 68.

———, and Hutt, F. B.: 1949 Environmental factors that do not influence the incidence of avian leucosis (Abstract). Poultry Sci. 28:761.

———, and Hutt, F. B.: 1951. Evidence that eggs do not transmit leucosis. Poultry Sci. 30.205.

Coles, J. D. W. A., and Bronkhorst, J. J.: 1946. The familial incidence of spontaneous osteopetrosis gallinarum. Onderstepoort Jour. Vet. Sci. and Anim. Ind. 21:79.

Cottral, G. E.: 1949. Avian lymphomatosis, another egg-borne disease. Proc. 53rd Ann. Meet. U.S. Livestock Sanit. Assn. 183.

———: 1952. The enigma of avian leukosis. Proc. 89th Ann. Meet. Am. Vet. Med. Assn., p. 285.

———, Burmester, B. R., and Waters, N. F.: 1949. The transmission of visceral lymphomatosis with tissues from embryonated eggs and chicks from "normal" parents (Abstract). Poultry Sci. 28:761.

———, and Winton, B.: 1953. Paralysis in ducks simulating neural lymphomatosis in chickens. Poultry Sci. 32:585.

Crispins, C. G., Sr.: 1960. Methylcholanthrene induced tumors in chickens. Poultry Sci. 39.1510.

Crittenden, L. B., Okazaki, W., and Reamer, R.: 1963. Genetic resistance to Rous sarcoma virus in embryo cell cultures and embryos. Virology 20.541.

Dalling, T., and Warrad, C. J.: 1936. Observations on fowl paralysis (lymphomatosis). Vet. Jour. 92:310.

Darcel, C. le Q.: 1950. Studies on the avian leukosis complex. 2. Some observations on the behaviour of three transplantable avian lymphoid tumours. Jour. Comp. Path. and Therap. 63:112.

———: 1953. Studies on the avian leukosis complex. Jour. Comp. Path. and Therap. Canad. Jour. Comp.

———: 1957. A note on the classification of the leucotic diseases of the fowl. Canad. Jour. Comp. Med. and Vet. Sci. 21:145.

Davis, O. S., Andrews, F. N., and Doyle, L. P.: 1950. Studies in avian leucosis. V. An investigation of the possible relationship of sex hormones to visceral lymphomatosis. Am. Jour. Vet. Res. 11:428.

Davis, O. S., and Doyle, L. P.: 1947. Studies in avian leucosis. I. The transmissibility of visceral lymphomatosis. II. The use of biopsy technique in the study of visceral lymphomatosis. Am. Jour. Vet. Res. 8:105.

———, and Doyle, L. P.: 1949. Studies in avian leucosis. IV. Further transmission of visceral lymphomatosis. Am. Jour. Vet. Res. 10:85.

———, Doyle, L. P., Walkey, F. L., and Cenker, L. K.: 1947. Studies in avian leukosis. III. The incidence of avian leukosis in various breeds of chickens. Poultry Sci. 26:499.

De Boer, E.: 1934a. Neurolymphomatosis gallinarum I. Tijdschr. Diergeneesk. 61:449.

———: 1934b. Neurolymphomatosis gallinarum II. Tijdschr. Diergeneesk. 61:520.

Denningsen, E. M., and Lucas, A. M.: 1960. Influence of heat treatment on the number of ectopic lymphoid foci in chickens. Am Jour. Vet. Res. 21:734.

DeOme, K. B.: 1945. Intraperitoneal injection of lymphomatous nerve tissue into resistant and susceptible chickens Poultry Sci 22:584

de Thé, G., Heine, U., Ishiguro, H., Sommer, J. R., Beard, D., and Beard, J. W.: 1962. Biologic response of nephrogenic cells to avian myeloblastosis virus. Federation Proceedings 21:919.

Deutsch, K., and Siller, W. G.: 1961 An electron microscopical study of the peripheral nerves in two cases of fowl paralysis (neurolymphomatosis). Res. Vet. Sci. 2:19.

Doan, C. A., Cunningham, R. S., and Sabin, F. R.: 1925. Experimental studies on the origin and maturation of avian and mammalian red and blood cells. Carnegie Inst. of Wash., Publ. 85:165.

Dobson, N.: 1934. Some poultry diseases. Vet. Record 14:532.

Doyle, L. P.: 1926. Neuritis in chickens. Jour. Am. Vet. Med. Assn. 68:622.

———: 1928 Neuritis or paralysis in chickens. Jour. Am. Vet. Med. Assn. 72:585.

Duran-Reynals, F. 1940. A hemorrhagic disease occurring in chicks inoculated with the Rous and Fujinami viruses. Yale Jour. Biol. and Med. 13:77.

———: 1941 Age susceptibility of chicks to the virus of the Rous sarcoma and variation of the virus in the dark. Science 93:501

———: 1947. A study of three new chick variants of the Rous chicken sarcoma. Cancer Res. 7:99.

Durant, A. J., and McDougle, H. C: 1939. Studies on the origin and transmission of fowl paralysis (neurolymphomatosis) by blood inoculation. Mo. Agr. Exper. Sta., Res. Bul. 304.

———, and McDougle, H. C: 1945. Further investigations of the transmission of fowl paralysis (neurolymphomatosis) by direct transfusion. Mo. Agr. Exper. Sta., Bul. 393:2.

Eckert, E. A., Beard, D., and Beard, J W: 1951 Dose-response relations in experimental transmission of avian erythromyeloblastic leukosis. I. Host-response to the virus. Jour. Nat. Cancer Inst 12:447.

———, Beard, D, and Beard, J W 1953 (With an appendix on histopathology by B. R. Burmester) Dose-response relations in experimental transmission of avian erythromyeloblastic leukosis II. Host response to whole blood and to washed primitive cells. Jour. Nat. Cancer Inst. 13:1167

———, Beard, D, and Beard, J. W.: 1956 Virus of avian erythroblastosis. I. Titration of infectivity. Jour. Nat Cancer Inst. 16.1099

———, Rott, R., and Schäfer, W: 1963 Myxovirus-like structure of avian myeloblastosis virus. Ztschr. f Naturforschung 18b 339

Edelson, L.' 1940. Private communication to Dr A. D. Goldhaft, Vineland Poultry Laboratories, Vineland, N. J.

Ellermann, V: 1920. Histogenese der übertragbaren Huhnerleukose. I. Die myeloische Leukose. Folia Haematol. 26:185.

———: 1921. Histogenese der übertragbaren Huhnerleukose. II. Die intravaskuläre lymphoide Leukose. Folia Haematol. 26:165.

———: 1922 The leucosis of fowls and leucemia problems. Cyldendal, London. P. 105.

———: 1923. Histogenese der übertragbaren Huhner Leukose IV. Zusammenfassende Betrachtungen. Folia Haematol. 29.203.

———, and Bang, O: 1908 Experimentelle Leukämie bei Huhnern. Zentralbl f. Bakt. Abt. I. Orig. 46.595.

Ellis, E. C, and Winn, J. D.: 1950. Unpublished data. July.

Emmel, M. W.: 1939. Hemocytoblastosis in the chicken. Proc. Seventh World's Poultry Cong, p. 298.

Engelbreth-Holm, J.: 1931. Bericht über einen neuen Stamm Huhnerleukose. Zeitschr. f. Immunitatsforsch u Exper. Therap 73 126.

———: 1932 Untersuchungen über die sogennante Erythroleukose bei Huhnern. Zeitschr. f. Immunitatsforsch u. Exper. Therap. 75:425.

———: 1942 Spontaneous and Experimental Leukaemia in Animals. Oliver and Boyd, London.

———, and Rothe-Meyer, A: 1932 II Über den Zusammenhang zwischen den verschiedenen Huhnerleukoseformen (Aname-Erythroblastose-Myelose). Acta Path. et Microbiol. Scand. 9 312.

———, and Rothe-Meyer, A.: 1935 On the connection between erythroblastosis haemocytoblastosis), myelosis, and sarcoma in chicken. Acta Path. et Microbiol. Scand. 12:352.

Eyestone, W. H.' 1950. The behavior of a transmissible lymphoid tumor in the tissues of chickens inbred for resistance and susceptibility to spontaneous lymphomatosis. Thesis. Univ. of Wisconsin.

Faddoul, G. P., and Ringrose, R. C: 1950. Avian keratoconjunctivitis. Vet. Med. 45:492.

Feldman, W. H.: 1932. Neoplasms of Domesticated Animals. W. B. Saunders Co., Philadelphia. Pp. 221–46.

———, and Olson, C., Jr.: 1933. The pathology of spontaneous leukosis of chickens. Jour. Am. Vet. Med. Assn. 82:875.

Findlay, G. M., and Wright, I.: 1935. Ocular lesions in epidemic blindness of fowls. Jour. Comp. Path. and Therap. 46:139.

Foulds, L.: 1934. The filterable tumours of fowls: A critical review. Sci. Rep. Invest. Imp. Cancer Res. Fund 11 (Suppl.). Pp. 1–41.

Fritsche, K.: 1938. Versuche zur Erforschung und Bekämpfung der Mareksehen Huhnerlähme. 1. Versuche uber die Ausscheidung des Huhnerlähmevirus mit dem Kot und über die natürliche Infektionsweise. Zeitschr. Infekt-Krankh. der Haustiere 52:51.

———: 1963. Experimentelle Untersuchungen über Osteopetrosis der Huhner. Proc. Seventeenth World's Vet. Cong. 2:1397.

Furth, J.: 1931a. Observations with a new transmissible strain of the leucosis (leucemia) of fowls. Jour. Exper. Med. 53:243.

———: 1931b. Erythroleukosis and the anemias of the fowl. Arch. Path. 12:1.

———: 1932a. Studies on the nature of the agent transmitting leucosis of fowls. I. Its concentration in blood cells and plasma and relation to the incubation period. Jour. Exper. Med. 55:465.

———: 1932b. Studies on the nature of the agent transmitting leucosis of fowls. III. Resistance to desiccation, to glycerin, to freezing, and thawing; survival at ice box and incubator temperatures. Jour. Exper. Med. 55:495.

———: 1933. Lymphomatosis, myelomatosis, and endothelioma of chickens caused by a filtrable agent. I. Transmission experiments. Jour. Exper. Med. 58:253.

———: 1934. Lymphomatosis, myelomatosis and endothelioma of chickens caused by a filtrable agent. II. Morphological characteristics of the endotheliomata caused by this agent. Jour. Exper. Med. 59:501.

———: 1936a. The relation of leukosis to sarcoma of chickens. II. Mixed osteochondrosarcoma and lymphomatosis (Strain 12). Jour. Exper. Med. 63:127.

———: 1936b. The relation of leukosis to sarcoma of chickens. III. Sarcomata of strains 11 and 13 and their relation to leukosis. Jour. Exper. Med. 63:145.

———: 1946. Recent experimental studies on leukemia. Physiol. Rev. 26:47.

———, and Breedis, C.: 1931. On the resistance and filterability of the agent transmitting leucosis. Proc. Soc. Exper. Biol. and Med. 28:985.

———, with assist. of Breedis, C.: 1935. Lymphomatosis in relation to fowl paralysis. Arch. Path. 20:379.

Gibbs, C. S.: 1934. Preliminary studies on neurolymphomatosis and some more or less related diseases. Mass. Agr. Exper. Sta., Bul. 308.

———, and Johnson, C. G.: 1935. Leukosis and avian paralysis. Mass. Agr. Exper. Sta., Bul. 315.

———, and Johnson, C. G.: 1936. Differentiation of the pathological cell in neurolymphomatosis from lymphocytes of the blood of chickens. The differentiation of neurolymphomatosis from lympholeukosis. Mass. Agr. Exper. Sta., Bul. 327.

Gildow, E. M., Williams, J. K., and Lampman, C. E.: 1940. The transmission of and resistance to fowl paralysis (lymphomatosis). Idaho Agr. Exper. Sta., Bul. 235.

Glick, Bu.: 1963. Possible cytoplasmic change in an immunologically competent tissue of the chicken. Science 142:485.

Gloster, R. E.: 1910. Fowl paralysis. Transmission of infective agent to young chickens. Vet. Jour. 96:427.

Goltz, W.: 1934a. Über die Wirkung arteigener Knochen- und Knochenmarkzellaststoffe auf die Knochen- und Blutbildung der Huhner. (Experimentell bei Huhnern erzeugte Osteodystrophia fibrosa, myelotische Leukose, Erythrämie und schwere Anämie) Frankf. Zeitschr. f. Path. 46:453.

———: 1934b. Knochenveränderungen bei experimentell bei Huhnern erzeugter Osteodystrophia fibrosa. Frankf. Zeitschr. f. Path. 47:63.

Gordon, R. F., Coles, R., and Stacey, G.: 1953. A transmission experiment with neurolymphomatosis Vet. Record. 67:297

Goodon, W. A. M.: 1960. Leucosis and fowl paralysis. Vet. Record 72:964.

Gray, E.: 1938. Pathogenic organisms and fowl paralysis. Vet. Record 50:1051.

———: 1910. Some experiments upon the therapeutic treatment of fowl paralysis (lymphomatosis) of poultry and the value of iodine in relieving the symptoms of such cases. Vet. Jour 96:28.

Greenwood, A. W., and Carr, J. G.: 1931. A possible connexion between the Rous 1 sarcoma virus and fowl paralysis. Official Rep., Ninth World's Poultry Cong., Paris. 3 9.

Gross, M. A.: 1957. The artificial and natural transmission of avian visceral lymphomatosis Southwestern Vet. 10 288

———, Burmester, B. R., and Mamel, N.: 1962. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. IV. Virus bioassay based on a logexponential distribution of host mortality times. Jour. Nat. Cancer Inst. 28.1111.

———, Burmester, B. R., and Walter, W. G.: 1959. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. I. Nature of the lesions. Jour. Nat. Cancer Inst. 22 83

Davis, O. S., and Doyle, L. P.: 1947. Studies in avian leucosis. I. The transmissibility of visceral lymphomatosis. II. The use of biopsy technique in the study of visceral lymphomatosis. Am. Jour. Vet. Res. 8:103.

——, and Doyle, L. P.: 1949. Studies in avian leucosis. IV. Further transmission of visceral lymphomatosis. Am. Jour. Vet. Res. 10:85.

——, Doyle, L. P., Walkey, E. L., and Cenker, L. K.: 1947. Studies in avian leukosis. III. The incidence of avian leukosis in various breeds of chickens. Poultry Sci. 26:499.

De Boer, E.: 1934a. Neurolymphomatosis gallinarum I. Tijdschr. Diergeneesk. 61:419.

—— 1934b Neurolymphomatosis gallinarum II. Tijdschr. Diergeneesk. 61:549.

Dennington, E. M., and Lucas, A. M.: 1960. Influence of heat treatment on the number of ectopic lymphoid foci in chickens. Am. Jour. Vet. Res. 21:754.

DeOme, K. B.: 1943. Intraperitoneal injection of lymphomatosis nerve tissue into resistant and susceptible chickens. Poultry Sci. 22:331.

de Thé, G., Heine, U., Ishiguro, H., Sommer, J. R, Beard, D., and Beard, J. W.: 1962. Biologic response of regenerative cells to avian myeloblastosis virus. Federation Proceedings 21:919.

Deutsch, K., and Siller, W. G.: 1961. An electron microscopical study of the peripheral nerves in two cases of fowl paralysis (neurolymphomatosis). Res. Vet. Sci. 2:19.

Doan, C. A, Cunningham, R. S, and Sabin, F. R.: 1925. Experimental studies on the origin and maturation of avian and mammalian red blood cells. Carnegie Inst. of Wash., Publ. 83:165.

Dobson, N.: 1934. Some poultry diseases. Vet. Rec. 14:352.

Doyle, L. P. 1926 Neuritis in chickens. Jour. Am. Vet. Med. Assn. 68:622.

—— 1928. Neuritis or paralysis in chickens. Jour. Am. Vet. Med. Assn. 72:585.

Duran-Reynals, F. 1940. A hemorrhagic disease occurring in chicks inoculated with the Rous and Fujinami viruses. Yale Jour. Biol. and Med. 13:77.

—— 1941. Age susceptibility of ducks to the virus of the Rous sarcoma and variation of the virus in the duck. Science 93:501.

—— 1947. A study of three new duck variants of the Rous chicken sarcoma. Cancer Res. 7:99.

Durant, A. J., and McDougle, H. C.: 1939. Studies on the origin and transmission of fowl paralysis (neurolymphomatosis) by blood inoculation. Mo. Agr. Exper. Sta., Res. Bul. 304.

——, and McDougle, H. C.: 1945 Further investigations of the transmission of fowl paralysis (neurolymphomatosis) by direct transfusion. Mo. Agr. Exper. Sta., Bul. 393.2.

Eckert, E. A., Beard, D., and Beard, J. W.: 1951. Dose response relations in experimental transmission of avian erythromyeloblastic leukosis. I. Host-response to the virus. Jour. Nat. Cancer Inst 12.447

——, Beard, D, and Beard, J W 1953 (With an appendix on histopathology by B. R. Burmester) Dose response relations in experimental transmission of avian erythromyeloblastic leukosis II Host-response to whole blood and to washed primitive cells. Jour. Nat. Cancer Inst 13.1167

——, Beard, D, and Beard, J W: 1956 Virus of avian erythroblastosis. I. Titration of infectivity. Jour. Nat. Cancer Inst. 16:1099

——, Rott, R, and Schäfer, W: 1963 Myxovirus-like structure of avian myeloblastosis virus. Ztschr f Naturforschung 18b*339

Edelken, I: 1940. Private communication to Dr. A D. Goldhaft, Vineland Poultry Laboratories, Vineland, N J

Ellermann, V: 1920 Histogenese der übertragbaren Huhnerleukose. I. Die myeloische Leukose. Folia Haematci. 26.135.

—— 1921. Histogenese der übertragbaren Huhnerleukose. II. Die intravaskuläre lymphoide Leukose. Folia Haematol. 26.165.

—— 1922. The leucoses of fowls and leucemia problems. Gyldendal, London. P. 105.

—— 1923 Histogenese der übertragbaren Huhnerleukose. IV. Zusammenfassende Betrachtungen. Folia Haematol. 29.203.

——, and Bang, O: 1908. Experimentelle Leukämie bei Huhnern. Zentralbl. f. Bakt. Abt. I. Orig 46 595.

Ellis, E. C, and Winn, J D: 1950. Unpublished data. July.

Emmel, M W: 1939 Hemocytoblastosis in the chicken. Proc. Seventh World's Poultry Cong, p.298.

Engelbreth-Holm, J: 1931. Bericht über einen neuen Stamm Huhnerleukose. Zeitschr. f. Immuntatsforsch. u. Exper. Therap. 73:126.

—— 1932 Untersuchungen über die sogenannte Erythroleukose bei Huhnern. Zeitschr. f. Immuntatsforsch. u. Exper. Therap. 75.425.

—— 1942. Spontaneous and Experimental Leukaemia in Animals. Oliver and Boyd, London

——, and Rothe-Meyer, A: 1932 II. Über den Zusammenhang zwischen den verschiedenen Huhnerleukoseformen (Anaemic-Erythroblastose-Myelose). Acta Path. et Microbiol. Scand. 9:312.

——, and Rothe Meyer, A: 1935 On the connection between erythroblastosis haemocytoblastosis), myelosis, and sarcoma in chicken. Acta Path. et Microbiol. Scand. 12:352.

Eyestone, W. H: 1950 The behavior of a transmissible lymphoid tumor in the tissues of chickens inbred for resistance and susceptibility to spontaneous lymphomatosis. Thesis. Univ. of Wisconsin.

Faddoul, G. P, and Ringrose, R. C: 1950. Avian keratoconjunctivitis. Vet. Med. 45:492.

Feldman, W. H.: 1932. Neoplasms of Domesticated Animals W. B Saunders Co., Philadelphia. Pp. 221–46.

———, and Olson, C., Jr.: 1933. The pathology of spontaneous leukosis of chickens. Jour. Am. Vet. Med. Assn. 82:875.

Findlay, G. M., and Wright, J.: 1933. Ocular lesions in epidemic blindness of fowls. Jour Comp Path. and Therap. 46:159.

Foulds, L.: 1934. The filterable tumours of fowls: A critical review Sci. Rep. Invest Imp. Cancer Res. Fund 11 (Suppl.). Pp. 1–41.

Fritzsche, K.: 1938. Versuche zur Erforschung und Bekämpfung der Mareckschen Hühnerlähme. I. Versuche über die Ausscheidung des Hühnerlähmevirus mit dem Kot und über die natürliche Infektionsweise. Zeitschr. Infekt-Krankh. der Haustiere 52:51.

———: 1963. Experimentelle Untersuchungen über Osteopetrosis der Hühner. Proc. Seventeenth World's Vet. Cong. 2:1397.

Furth, J.: 1931a. Observations with a new transmissible strain of the leucosis (leucemia) of fowls. Jour. Exper. Med. 53:243.

———: 1931b. Erythroleukosis and the anemias of the fowl. Arch. Path. 12:1.

———: 1932a. Studies on the nature of the agent transmitting leucosis of fowls 1 Its concentration in blood cells and plasma and relation to the incubation period. Jour. Exper. Med. 55:465.

———: 1932b. Studies on the nature of the agent transmitting leucosis of fowls, III. Resistance to desiccation, to glycerin, to freezing, and thawing; survival at ice box and incubator temperatures. Jour. Exper. Med. 55:495.

———: 1933. Lymphomatosis, myelomatosis, and endothelioma of chickens caused by a filtrable agent. I. Transmission experiments. Jour. Exper. Med. 58:253.

———: 1934. Lymphomatosis, myelomatosis and endothelioma of chickens caused by a filtrable agent. II. Morphological characteristics of the endotheliomata caused by this agent. Jour. Exper. Med. 59:501.

———: 1936a. The relation of leukosis to sarcoma of chickens. II. Mixed osteochondrosarcoma and lymphomatosis (Strain 19). Jour. Exper. Med. 63:127.

———: 1936b. The relation of leukosis to sarcoma of chickens. III. Sarcomata of strains 11 and 15 and their relation to leukosis. Jour. Exper. Med. 63:145.

———: 1946. Recent experimental studies on leukemia. Physiol. Rev. 26:47.

———, and Breedis, C.: 1931. On the resistance and filterability of the agent transmitting leucosis. Proc. Soc. Exper. Biol. and Med. 28,985.

———, with assist. of Breedis, C.: 1935. Lymphomatosis in relation to fowl paralysis. Arch. Path. 20:379.

Gibbs, C. S.: 1934. Preliminary studies on neurolymphomatous diseases, Mass. Agr. Exper. Sta., Bul. 308.

———, and Johnson, C. G.: 1935. Leukosis and avian paralysis. Mass. Agr. Exper. Sta., Bul. 315.

———, and Johnson, C. G.: 1936. Differentiation of the pathological cell in neurolymphomatosis from lymphocytes of the blood of chickens. The differentiation of neurolymphomatosis from lympholeukosis. Mass. Agr. Exper. Sta., Bul. 327.

Gildow, E. M., Williams, J. K., and Lampman, C. E.: 1940. The transmission of and resistance to fowl paralysis (lymphomatosis). Idaho Agr. Exper. Sta., Bul. 235.

Glick, B.: 1963. Possible cytotoxic change in an immunologically competent tissue of the chicken. Science 142:486.

Glover, R. E.: 1940. Fowl paralysis Transmission of infective agent to young chickens. Vet. Jour. 96:427.

Gohs, W.: 1934a. Über die Wirkung artigener Knochen- und Knochenmarkezerfallstoffe auf die Knochen- und Blutbildung der Hühner. (Experimentell bei Hühnern erzeugte Osteodystrophia fibrosa, myeloische Leukose, Erythrämie und schwere Anämie.) Frankf. Zeitschr. f. Path. 46:453.

———: 1934b. Knochenveränderungen bei experimentell bei Hühnern erzeugter Osteodystrophia fibrosa. Frankf. Zeitschr. f. Path. 47.63.

Gordon, R. F., Coles, R., and Stacey, C. G.: 1955 A transmission experiment with neurolymphomatosis. Vet. Record. 67:297.

Gordon, W. A. M.: 1960. Leucosis and fowl paralysis. Vet. Record 50:1051.

Gray, G.: 1938. Pathogenic organisms and fowl paralysis (lymphomatosis). Vet. Record 72:964.

———: 1940 Some experiments upon the therapeutic treatment of fowl paralysis (lymphomatosis) of poultry and the value of iodine in relieving the symptoms of such cases. Vet. Jour 96.28.

Greenwood, A. W., and Carr, J. G.: 1951. A possible connexion between the Rous 1 sarcoma virus and fowl paralysis. Official Rep., Ninth World's Poultry Cong., Paris. 3.9.

Gross, M. A.: 1957. The artificial and natural transmission of avian visceral lymphomatosis. Southwestern Vet. 10.288

Burmester, B. R., and Mantel, N.: 1962. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. IV. Virus bioassay based on a logexponential distribution of host mortality times. Jour. Nat. Cancer Inst. 28 1111.

———, Burmester, B. R., and Walter, W. G.: 1959. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. 1 Nature of the lesions. Jour. Nat. Cancer Inst. 22 83.

Hall, W. J., Bean, C. W., and Pollard, M.: 1941. Transmission of fowl leukosis through chick embryos and young chicks. Am. Jour. Vet. Res. 2:272.
———, and Pollard, M.: 1943. Further studies on the propagation of fowl leucosis in chick embryos by intravenous inoculation. Am. Jour. Vet. Res. 4:287.
Hamilton, C. M., and Sawyer, C. E.: 1939. Transmission of erythroleukosis in young chickens. Poultry Sci 18:388.
Hamilton, H P.: 1934. Fowl paralysis, a disclaimer. Vet. Record 14:416.
Hanafusa, H., Hanafusa, T., and Rubin, H.: 1963. The defectiveness of Rous sarcoma virus. Proc. Nat. Acad. Sci. 49:572.
Harris, S. T.: 1939. Lymphomatosis (fowl paralysis) in the pheasant. Vet. Jour. 95:104.
———, Johnston, J. W., and Mitchell, S. C. A.: 1947. A study of isolation in fowl paralysis (lymphomatosis). Vet. Jour. 103 304.
Heine, U, de Thé, G., Beard, J., and Beard, J. W.: 1963. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. V. Elaboration of virus by pancreas of chickens inoculated with the agent. Jour. Nat. Cancer Inst. 30:817.
Heisdorf, A J., Brewer, N. R., and Lamoreux, W. F.: 1947. The genetic relationship between mortality from induced and spontaneous lymphomatosis. Poultry Sci. 26.67.
Helmboldt, C F. and Frazier, M. N.: 1962. Neurofibromatosis in a turkey. Jour. Am. Vet. Med. Assn 141 1073
———, Wills, F K., and Frazier, M. N.: 1963. Field observations of the pathology of skin leukosis in Gallus gallus. Avian Dis. 7:402.
Hepding, L.: 1939 Ueber Toxoplasmen (Toxoplasma gallinarum n. sp.) in der Retina eines Huhnes und über deren Beziehung zur Huhnerlähmung. Zeitschr. f. Infkt-Krankh, der Haustiere 55 109.
Holmes, J. R.: 1958. Experimental transmission of avian osteopetrosis. Jour. Comp. Path. and Therap. 68:439.
———: 1959 Further studies on the experimental transmission of avian osteopetrosis. Jour. Comp Path. and Therap. 69 385
———: 1961. Postmortem findings in avian osteopetrosis. Jour. Comp, Path. and Therap. 71.20
Horiuchi, T.: 1961. Pathological studies on avian visceral lymphomatosis, especially on gross and histopathology of liver and spleen. Japanese Jour. Vet. Sci. 23:227.
Hughes, W. F., Watanabe, D. H., and Rubin, H.: 1963. The development of a chicken flock apparently free of leukosis virus. Avian Dis. 7:154.
Hutt, F. B.: 1932 Light line new mutations in the domestic fowl. Proc. Sixth Internat. Cong. Genetics 2 96
———: 1939. Breeding strains of poultry resistant to fowl paralysis. Harper-Adams. Util. Poultry Jour. 24.995.
———: 1944. Simplified control of fowl leucosis. Farm Res. N.Y. St. and Cornell Sta. 10:11.
———: 1945. The high cost of fowl leucosis. Jour. Am. Vet. Med. Assn. 106:174.
———: 1951. The control of leucosis in the fowl. World's Poultry Sci. Jour. 7:116.
———, and Cole, R. K.: 1947. The comparative importance of genes and of supposed egg-borne agents in the etiology of avian lymphomatosis. (Abst.) Poultry Sci. 26.544.
———, and Cole, R. K.: 1948 The development of strains genetically resistant to avian lymphomatosis. Official Rep., Eighth World's Poultry Cong. Copenhagen: 719.
———, and Cole, R. K.: 1954 Problems concerning leucosis and its control. Proc. 10th World's Poultry Cong, Edinburgh, Scotland: Part 2, 197.
———, and Cole, R. K.: 1955. Resistance to lymphomatosis in the fowl in relation to reproduction. Nature 176:1178
———, Cole, R. K., Ball, M., Bruckner, J. H., and Ball, R. F.: 1944. A relation between environment to two weeks of age and mortality from lymphomatosis in adult fowls. Poultry Sci. 23 596.
———, Cole, R. K., and Bruckner, J. H.: 1941. Four generations of fowls bred for resistance to neoplasms. Poultry Sci. 20.514.
Ishiguro, H, Beard, D., Sommer, J. R., Heine, U, de Thé, G., and Beard J. W.: 1962. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. I. Nephroblastoma (Wilm's tumor). Gross and microscopic pathology. Jour. Nat. Cancer Inst. 29:1.
Ishitani, R.: 1957. Pathological studies on avian erythroleucosis. Nat. Inst. of Anim. Health, Tokyo, Bul. 32.261.
Jaensch, P. A., and Lerche, G.: 1933. Die Augenveränderungen bei Marekscher Geflugellähme. Graefes Arch. f Ophthalm. 131:359.
Jármai, K.: 1930. Beiträge zur Kenntnis der Huhnerleukose. Arch. f. wiss. u. prakt. Tierheilk. 62.113.
———: 1933. Infektionsversuche bebruteter Eier mit dem "Virus" der Huhnererythroleukose. Deutsch. tierärtzl. Wochenschr. 41:418.
———: 1934 Die Leukosen der Haustiere Ergeb. der Allg Path, Mensch. u. Tiere. 28.277.
———: 1935. Tumorerzeugung mit dem Leukosagens der Huhner. Arch. f. wiss. u. prakt. Tierheilk. 69.275.
———, Stenszky, T., and Farkas, L.: 1932. Neuere Beitrage zur Kenntnis der ubertragbaren Huhnerleukose Arch f. wiss u. prakt. Tierheilk. 65:46.

Johnson, E. P.: 1932. A study of lymphomatosis of fowls. Va. Agr. Exper. Sta., Tech. Bul. 44.
———: 1934. The etiology and histogenesis of leucosis and lymphomatosis of fowls. Va. Agr. Exper. Sta., Tech. Bul. 56.
———: 1937. Transmission of fowl leukosis. Poultry Sci. 16 255.
———: 1941. Fowl leukosis—manifestations, transmission, and etiological relationship of various forms. Va. Agr. Exper. Sta., Tech. Bul. 76.
———: 1945. Experimental vaccination for prevention of the avian leucosis complex. Am. Jour. Vet. Res. 6:198.
———, and Conner, B. V.: 1933. Blood studies of fowls with various forms of lymphomatosis (fowl paralysis). Jour. Am. Vet. Med. Assn. 83:325.
Jones, E. E.: 1934. Epidemic tremor, an encephalomyelitis affecting young chickens. Jour. Exper. Med. 59:781.
Jordan, H. E.: 1936. The relation of lymphoid tissue to the process of blood production in avian bone marrow. Am. Jour. Anat. 59:249.
———, and Johnson, E. P.: 1935. Erythrocyte production in the bone marrow of the pigeon. Am. Jour. Anat. 56:71.
Joyet, P. E.: 1954. The peroxidases of avian leucocytes. Proc. 10th World's Poultry Cong., Edinburgh, Scotland, 2:206.
Jungherr, E.: 1933. Observations on the macroscopic diagnosis of fowl paralysis. Poultry Sci 12:184.
———: 1934. Studies on fowl paralysis. 1. Diagnosis. Storrs Agr. Exper. Sta., Bul. 200.
———: 1936. The etiologic and diagnostic aspects of the fowl paralysis problem. Jour. Am. Vet. Med. Assn. 86:424.
———: 1937. Studies on fowl paralysis. 2. Transmission experiments. Storrs Agr. Exper. Sta., Bul. 218.
———: 1939. Neurolymphomatosis phasianorum. Jour. Am. Vet. Med. Assn. 04:49.
———: 1940. Wheat germ oil in the control of fowl paralysis. Poultry Sci. 19:94.
———: 1953. Neuropathologic differentiation of symptomatic paralysis in fowl. Proc. 15th Internat. Vet. Cong. Stockholm, P. 1., 2:1062.
———, Doyle, P., and Johnson, E. P.: 1941. Tentative pathologic nomenclature for the disease and/or for the disease complex variously designated as fowl leucemia, fowl leucosis, etc. Am. Jour. Vet. Res. 2:116.
———, and Landauer, W.: 1938. Studies on fowl paralysis. 3. A condition resembling osteopetrosis (Marble Bone) in common fowl. Storrs Agr. Exper. Sta., Bul. 222.
———, and Wolf, A.: 1939. Gliomas in animals. A report of two astrocytomas in the common fowl. Am. Jour. Cancer 37:493.
Kaupp, B. F.: 1921. Paralysis of the domestic fowl. Jour. Am. Assn. of Instructors and Invest. in Poultry Husb. 7:25.
Kennard, D. C., and Chamberlin, V. D.: 1934. Poultry mortality. Ohio Agr. Exper. Sta., Bimo Bul. 19:137-42. (Bul. 169.)
Kenzy, S. G.: 1955. Studies in avian neoplasia. 1. A quantitative evaluation of neutralizing antibodies for Rous sarcoma virus in avian serums. Am. Jour. Vet. Res. 13.588.
———, and Neuzil, P. V.: 1955. Incidence of Rous virus neutralizing antibodies in serums collected from flocks experiencing losses due to lymphomatosis. Am. Jour. Vet. Res. 14:123.
Kissling, R. E.: 1947. Leukoagglutination as a serological diagnosis for avian lymphomatosis. Poultry Sci. 26:74.
Kitt, T.: 1931. Die Leukomyelose der Huhner. Ergeb. der Hyg., Bakt. Immunitatsforsch. u. exper Therap. 12:15.
Kondo, M.: 1937. Die Entwicklung der Lymphknotchen am Lymphgefassystem des Huhnes. Folia Anat. Jap. 15:349.
Lagerlof, B., and Sundelin, P.: 1963a. The histogenesis and haematology of virus-induced myeloid leukaemia in the fowl. Acta Haematologica 30:111.
———, and Sundelin, P.: 1963b. Variations in the pathogenic effect of myeloid fowl leukaemia virus. Acta Path. Microbiol. Scand. 59:129.
Lee, G. D., and Wilcke, H. L.: 1941. Transmission experiments with ritis of fowls. Am. Jour. Vet. Res. 2:292.
Leitche, M., and Fritzsche, K.: 1934. Histopathologie und Diagnostik der Geflugellahme. Zeitschr. f. infekt-Krankh. d. Haustiere 45:89.
Lesher, S., and Burmester, B. R.: 1965. Plasma phosphatase activities of normal and lymphomatous chickens. Cancer Res. 15 537.
Levine, S., and Nelsen, D.: 1964. RIF infection in a commercial flock of chickens. Avian Dis. 8:358.
Lucas, A. M.: 1949. Lymphoid tissue and its relation to so called normal lymphoid foci and to lymphomatosis. 1. Qualitative study of lymphoid areas in the pancreas of chickens. Am Jour. Path. 25:1197.
———: 1951. VI. A study of lymphoid areas in the pancreas of dove and pigeons. Poultry Sci. 30.116.
———, and Bretmayer, J. B.: 1949. III. Qualitative and quantitative comparison of lymphoid areas in the pancreas of the White Pekin duck with those in chickens. Poultry Sci. 28:436.
———, and Bretmayer, J. B.: 1950. V. A study of lymphoid areas in the pancreas of pheasants and wild Mallard ducks. Poultry Sci. 29:450.

Lucas, A. M., Graig, C. C., Oakberg, E. F., and Breitmayer, J. B.: 1949. IV. Simplification of methods for quantitative analyses and its application to the turkey. Growth 13:339.

——, and Oakberg, E. F.: 1950. II. Quantitative analysis of lymphoid areas in the pancreas of laboratory and farm chickens. Am. Jour. Path. 26:75.

McClary, C. F., and Upp, C. W.: 1939. Is paralysis of fowls, as manifested by iritis, transmitted through the egg? Poultry Sci. 18:210.

McDaniel, 1 N , McDaniel, L. S., and Chute. H. L. 1964. Persistence of Rous sarcoma virus in the mosquito *Culex pipiens pipiens*. Am. Jour. Vet. Res. 25:262.

McDaniel, L. S., McDaniel, L. N., and Chute. H. L.: 1962. Laboratory transmission of Rous sarcoma virus by *Aedes aegypti*. Avian Dis. 6:127

McGaughey, C. A., and Downie, A. W: 1930. Preliminary report on an outbreak of fowl paralysis in England. Jour. Comp. Path and Therap. 43:63.

McIntosh, J.: 1933. On the nature of tumors induced in fowls by injections of tar. Bnt. Jour. Exper. Path 14:422

——, and Selbie, F R.: 1939. Further observations on filtrable tumors induced in fowls by injections of tar. Bnt Jour Exper. Path. 20:49

McKee, G S , Lucas, A. M., Denington, E M., and Love, F. C.: 1965. Separation of leukotic and non-leukotic lesions in turkeys on the inspection line. Avian Dis. 7:19.

McLennan, G. C.: 1935. "Range paralysis' *Neuro-lymphomatosis gallinarum*. Australian Vet. Jour. 11:42.

Magnusson, H.: 1935. Hönsparalys Svensk Vet Tidskr 40:43

——: 1946. Om en ovanlig sjukdomsbild hörande till hönsleuloskomplexet. Skand. Veterinärtidskrift för bakt., patol, samt kött-och mjölkhyg 36:70.

Marek, J.: 1907. Multiple Nervenentzundung (Polyneuritis) bei Hühnern. Deutsch. tierärztl. Wochenschr. 15:417.

Manne, D., and Rosen, S H : 1941 Sex hormones and lymphomatosis in fowls. Proc. Soc. Exper. Biol. and Med. 47:61

Mathews, F. P : 1929 Leukochloroma in the common fowl. Its relation to myelogenic leukemia and its analogies to chloroma in man Arch. Path. 7:442.

——, and Walker, F L : 1929 Lymphadenoma of the common fowl. Jour. Cancer Res. 13:383.

Moynihan, I W 1943 Avian osteopetrosis Canad. Jour, Comp. Med. and Vet. Sci. 7:327.

Munroe, J S , and Windle, W F 1963 Tumors induced in primates by chicken sarcoma virus. Science 140:1415.

Murphy, J B , and Sturm, E 1941a. Further investigation of induced tumors in fowls. Cancer I 477

——, and Sturm E 1941b Further investigations on the transmission of induced tumors in fowls. Cancer Res. 1:609.

Nelson, N M : 1947 Normal eye color in the chicken. Poultry Sci. 26:61.

——, and Thorp, F., Jr: 1945. Ocular lymphomatosis with special reference to chromatism of index lens Am Jour. Vet Res. 4:294

Newberne, P M., and Vosbrink, C. J.: 1958 The avian leukosis complex. Vet. Med. 53:685.

Noris, L. C., Caskey, C D., and Bauernfeind, J. C., 1940. Malformation of the tarso-metatarsal and phalangeal bones of chicks. Poultry Sci. 19:219.

Nyfeldt, A.: 1934 Étude sur les leucoses des poules. I. Une myéloblastose pure. Sang 8:566.

Oakberg, E F.: 1950. Distribution and amount of lymphoid tissue in some of the splanchnic nerves of chickens in relation to age, sex and individual constitution. Poultry Sci. 29:420.

——, and Lucas, A. M.: 1949a. Effect of age, sex and individual variability on lymphoid tissue of the pancreas in White Leghorn chickens Poultry Sci. 28:635.

——, and Lucas, A. M.: 1949b. Variations in body weight and organ: Body weight ratios of inbred lines of White Leghorn chickens in relation to mortality, especially from lymphomatosis Growth 13 819.

Oakley, C. L.: 1935. Lymphomatosis. Proc. Roy. Soc. Med. 28 999

Oberling, Ch., and Guérin, M.: 1933a. Lésions transmissibles en rapport avec la leucémie transmissible des poules Bul. Assoc. franc pour Étude Cancer 22:180.

——, and Guérin, M. 1933b. Nouvelles recherches sur la production de tumeurs malignes avec le virus de la leucémie transmissibles des poules. Bul. Assoc. franc. pour Étude Cancer 22:926.

——, and Guérin, M.: 1933c. Nouvelles recherches sur les ostéites par carence chez les poules. Compt. rend. Soc. de biol. 112:1288.

——, and Guérin, M.: 1934. La leucémie érythroblastique ou érythroblastose transmissible des poules. Bul. Assoc. franc, pour Étude Cancer 23 38.

——, and Guérin, M.: 1934 The role of viruses in the production of cancer. *Advances in Cancer Research*. II Academic Press, New York, N.Y., p. 353.

——, Guérin, M., and Guérin, N V : 1933. Recherches sur la leucémie transmissible (érythroblastose) des poules. Compt rend. Soc de biol 112:559

——, and Muller, J.: 1934. Tentatives d'homogreffes parathyroidiennes chez des poules carencées par un régime pauvre en calcium. Ann. Anat. Path. et Anat. Norm. Med.-Chir. 11:744.

Olson, C., Jr.: 1936. A study of transmissible fowl leukosis. Jour. Am. Vet. Med. Assn. 89:681.

——: 1937. Attempts to transmit fowl paralysis. Jour. Infect. Dis 61:325.

——: 1940. *Transmissible fowl leukosis*. A review of the literature. Mass. Agr. Exper. Sta , Bul. 370.

——: 1941. A transmissible lymphoid tumor of the chicken. Cancer Res. 1:384.

———: 1945a. The immunizing action of a lymphoid tumor in chickens. Am. Jour. Vet. Res. 6:103.

———: 1945b. Immunization against a lymphoid tumor of the chicken. I. Attenuation by freezing. Cornell Vet. 35:221.

———: 1947. Immunization against a lymphoid tumor of the chicken. IV. Use of miscellaneous tissues. Cornell Vet. 37:231.

———, Fredrickson, T. N., Tekeli, S., and Bird, H. R.: 1962. A dietary factor influencing lymphoid tumor of the chicken. Proc. Soc. Exper. Biol. and Med. 111:44.

———, and Zeissig, A.: 1936. A study of the antigenic properties of certain normal and pathological lymphoid deposits in tissues of chickens. Jour. Immunol. 31:309.

Pappenheimer, A. M., Dunn, L. C., and Cone, V.: 1926. A study of fowl paralysis (neurolymphomatosis gallinarum). Storrs Agr. Exper. Sta., Bul. 143.

———, Dunn, L. C., and Cone, V.: 1929a. Studies on fowl paralysis (neurolymphomatosis gallinarum). I. Clinical features and pathology. Jour. Exper. Med. 49:63.

———, Dunn, L. C., and Seidlin, S. M.: 1929b. Studies on fowl paralysis (neurolymphomatosis gallinarum). II. Transmission experiments. Jour. Exper. Med. 49:87.

Paterson, J. C., and Cottral, G. E.: 1950. Experimental coronary sclerosis III. Lymphomatosis as a cause of coronary sclerosis in chickens. Arch. Path. 49:699.

Patterson, F. D.: 1934. Neurolymphomatosis gallinarum. Proc. Twelfth Internat. Vet. Cong. (New York) 3:265.

———: 1936. Fowl leukosis. Jour. Am. Vet. Med. Assn. 88:32.

Wicke, H. L., Murray, Chas., and Henderson, E. W.: 1932. So-called range paralysis of the chicken. Jour. Am. Vet. Med. Assn. 81:747.

Peckham, M. C.: 1957. Case report — lens opacities in fowls possibly associated with epidemic tremors. Avian Dis. 1:247.

Pentimalli, F.: 1915. Über die Geschwulste bei Hühnern. I. Mitteilung. Allgemeine Morphologie der spontanen und der transplantablen Hühnergeschwülste. Zeitschr. f. Krebsforsch. 15:111.

———: 1941. Transplantable lymphosarcoma of the chicken. (Abst.) Cancer Res. 1:68.

Peterson, R. D. A., Burmester, B. R., Fredrickson, T. N., and Good, R. A.: 1963. Prevention of lymphatic leukemia in the chicken by the surgical removal of the bursa of Fabricius. Jour. Lab. and Clinical Med. 62:1000.

Phillips, P. H., and Engel, R. W.: 1938. The histopathology of neuromalacia and "curled toe" paralysis in the chick fed low riboflavin diets. Jour. Nutr. 16:451.

Piedrafita, J. G.: 1951. Transmission of hen leucosis through vaccines obtained from dead virus used against Newcastle disease. Official Rep., Ninth World's Poultry Cong., Paris. 3:146.

Pollard, M., and Hall, W. J.: 1941. Preliminary report on inter-species transmission of avian leucosis in embryo. Jour. Am. Vet. Med. Assn. 99.219 (Abst.).

Pontén, J., and Thorell, B.: 1957. The histogenesis of virus-induced chicken leukemia. Jour. Nat. Cancer Inst. 18:443.

Potel, K.: 1938. Hyalin-scholige Degeneration der Skeletmuskulatur bei Kücken und Jung-hühnern. Arch. f. wiss. u. prakt. Tierheilk. 72.216.

Pugh, L. P.: 1927. Sporadic diffuse osteo-petrositis of fowls. Vet. Record 7:189.

Purchase, C.: 1963. American Association of Avian Pathologists leukosis symposium, 100th Ann. Meet, AVMA, New York City, August.

Reinhardt, R.: 1930. Die pathologisch-anatomischen Veränderungen bei den spontanen Krankheiten der Hausvögel. Ergeb. d. allgem. Path. u. path. Anat. des Mensch. u. der Tiere. 23.553.

Reis, J., and Nobrega, P. (com. collab. Reis, A. S.): 1936. Doenças das Aves. (Tratado de ornitho-pathologia). Instituto Biologico, São Paulo, Brazil, p. 417.

Rigdon, R. H.: 1959. Cataracts in chickens with lymphomatosis. Am. Jour. Vet. Res. 20.647.

Ringoen, A. R.: 1934. The inter-sinusoidal capillaries of avian bone marrow. Anat. Record 58:84 (Suppl.).

Rothe-Meyer, A., and Engelbreth-Holm, J.: 1933. Experimentelle Studien über die Beziehungen zwischen Hühnerleukose und Sarkom an der Hand eines Stammes von übertragbarer Leukose-Sarkom Kombination. Acta. Path. et Microbiol. Scand. 10:380.

Rubin, H.: 1960. A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus. Proc Nat. Acad. Sci. 46:1105.

———, Cornelius, A., and Fanshier, L.: 1961. The pattern of congenital transmission of an avian leukosis virus. Proc. Nat. Acad. Sci. 47:1058.

———, Fanshier, L., Cornelius, A., and Hughes, W. F.: 1962. Tolerance and immunity in chickens after congenital and contact infection with an avian leukosis virus. Virology 17:143.

———, and Vogt, P. K.: 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. Virology 17:184.

Schmeisser, H. C.: 1915. Spontaneous and experimental leukemia of the fowl. Jour. Exper. Med. 22:820.

Seagar, E. A.: 1933. Cellular reactions in the blood in neuro-lymphomatosis gallinarum (fowl paralysis) Vet. Jour. 89:557.

Seifried, O., and Sassenhof, I.: 1940. Osteomyelosklerose bei Hühnern. Eine bei Haustieren bisher unbekannte, generalisierte Krankheit des Skelets. Arch. wiss. praht. Tierheilk. 75.411.

Seitne, F. R., and McIntosh, J.: 1939. Factors influencing the infectivity of fowl tumors. Brit. Jour. Exp. Path. 20:443.

Sevoian, M., and Chamberlain, D. M.: 1962. Avian lymphomatosis. II. Experimental reproduction of the ocular form Vet Med. 57:608

———, Chamberlain, D. M.: 1963. Avian lymphomatosis. III. Incidence and manifestations in experimentally infected chickens of various ages. Avian Dis. 7:97.

———, and Chamberlain, D. M. 1964. Avian lymphomatosis. IV. Pathogenesis. Avian Dis 8 281.

———, Chamberlain, D M., and Counter, F.: 1962. Avian lymphomatosis. I. Experimental reproduction of neural and visceral forms Vet Med. 57:500.

———, Chamberlain, D M., and Larose, R. N: 1963a. Avian lymphomatosis. VII. New support for etiologic unity. Proc Seventeenth World's Vet Cong. 2:1475.

———, Chamberlain, D M., and Larose, R. N.: 1963b Avian lymphomatosis. V. Airborne transmission Avian Dis 7 102

———, Larose, R N., and Chamberlain, D. M.: 1964. Avian lymphomatosis. VI. A virus of unusual potency and pathogenicity Avian Dis 8.336.

Sharpless, G R Davies, M C, and Cox, H R : 1950. Antagonistic action of certain neurotropic viruses toward a lymphoid tumor in chickens with resulting immunity. Proc. Soc. Exper Biol, and Med. 75 270

———, and Jungherr, E L 1961 Characterization of 2 viruses obtained from lymphomatous liver Am Jour Vet Res 22 937.

Siller, W G 1960 An unusual case of fowl paralysis. Jour. Comp. Path, and Therap. 70:156.

Simons, P J and Dougherty, R M 1963. Antigenic characteristics of three variants of Rous sarcoma virus. Jour, Nat Cancer Inst. 31:1275

Simpson, C F , Anthony, D W, and Young, F 1957. Visceral lymphomatosis in a flock of turkeys Jour Am. Vet Med Assn 130 93

Sjolte, I, P 1950 On Osteomyeloskerose hos Hons. Nordisk. Veterinær Med. 2:509.

Storti, E., and Mezzadra, G : 1930 Tentatives de culture du virus de la leucémie des poules dans la membrane chorio allantoide (Note préliminaire) Sang 12.533.

Stubbs, E. L 1938. Fowl leukosis. Jour. Am Vet Med. Assn. 92:73.

———, and Furth, J 1932 Anemia and erythroleucosis occurring spontaneously in the common fowl. Jour Am Vet Med. Assn, 81 209.

———, and Furth, J 1935. The relation of leukosis to sarcoma of chickens, I. Sarcoma and erythroleukosis (strain 13) Jour Exper. Med 61:593.

Stet-Moldavsky, G. J.: 1957. Development of multiple cysts and of haemorrhagic affections of internal organs in albino rats treated during the embryonic or newborn period with Rous sarcoma virus Nature (London) 180,1299.

———, and Skorikova, A S. 1960 The pathogenicity of Rous sarcoma virus for mammals. Detection of virus and of antigenic substances of Rous sarcoma in the cyst-haemorrhagic disease of albino rats. Acta Virologica (English ed.) 4,47.

Taylor, L W, and DeOme, K B 1959 Failure of wheat germ oil to prevent lymphomatosis in chickens. Jour Am. Vet. Med. Assn. 95 73.

———, Lerner, I M., DeOme, K B, and Beach, J. R.: 1943 Eight years of progeny-test selection for resistance and susceptibility to lymphomatosis. Poultry Sci. 22 339.

Thiersch, J., B 1944. Attempts to transmit leucaemia of man and of mice to the chick embryo and to the young chick by the amniotic and intravenous routes. Australian Jour. Exper. Biol. and Med Sci 22 57.

Thousen, O, and Engelbreth-Holm, J 1932 De la provocation expérimentale d'états leucosiformes chez les poules. Compt. rend Soc de biol. 109,1213

Thorbecke, G. J., Gordon, H. A., Wostman, B, Wagner, M , and Reyniers, J, A : 1957. Lymphoid tissue and serum gamma globulin in young germfree chickens Jour. Infect, Dis. 101:237,

Uhl, E : 1938. Active immunization of chickens against chicken leukosis with agent adsorbed by aluminum hydroxide Acta Path. et Microbiol, Scand. Suppl. 57:544.

U.S. Bureau of Animal Industry. 1951. Twelfth Ann Rep. of the Regional Poultry Research Laboratory. East Lansing, Michigan.

Upp, C., and Tower, B A.' 1936. The incidence of blindness and paralysis according to family. Poultry Sci. 15:421.

Van den Berghe, L., and d'Ursel, Fr.' 1939. Erythroblastose du poussin éclos après inoculation chorio allantoidienne du virus (souche O G')   Compt. rend, Soc. de biol. 131:1302.

Van der Walle, N., and Winkler-Junius, E., 1924. De Neuritis-Epizootie bij Kippen te Barneveld in 1921. Tijdschr. voor verg. Geneesk. 10.31.

Venkataraman, R: 1956. A note on osteitis deformans in two fowls. Indian Jour. Vet. Sci. 6 108.

Vindel, J. A.: 1960 Contribution a la connaissance de la neurolymphomatose aviaire. Ann. Zootech. 11:70

———: 1964. La neurolymphomatose aviaire. Epizootiologie, etiologie et pathogénie. Rec. Méd. Vét. 140 87

Walter, W. C , Burmester, B. R., and Cunningham, C. H.' 1962. Studies on the transmission and pathology of a viral-induced avian nephroblastoma (embryonal nephroma). Avian Dis. 6:455.

———, Burmester, B. R., and Fontes, A K. 1963. Variation in the occurrence of erythroblastosis and osteopetrosis induced by virus from individual chickens infected with avian leukosis strain RLP 12. Avian Dis. 7:79.

Warrack, G. H., and Dalling, T.: 1932. So-called fowl paralysis. Also called neuritis in chickens, range paralysis, *neurolymphomatosis gallinarum*. (A discussion on the various theories as to causation with special reference to field observations and laboratory transmission experiments.) Vet. Jour. 88.28.

Waters, N. F.: 1945a. Natural transmission of avian lymphomatosis. Poultry Sci. 24:226.

———: 1945b. Breeding for resistance and susceptibility to avian lymphomatosis. Poultry Sci. 24:259.

Waters, N. F.: 1945c. Lymphomatosis in chickens as influenced by diallel crossing. Poultry Sci. 24:387.

———: 1947. The contagious nature of a lymphoid tumor in chickens. Science 106.246.

———: 1951. Mortality from lymphomatosis and other causes among inbred lines of White Leghorns. Poultry Sci. 30.531.

———: 1954. Avian lymphomatosis mortality among inbred line crosses. Proc 10th World's Poultry Cong., Edinburgh, Scotland, Part 2:201.

———, and Burmester, B. R.: 1961. Mode of inheritance of resistance to Rous sarcoma virus in chickens. Jour. Nat. Cancer Inst. 27:655.

———, and Bywaters, J. H.: 1949. Influence of age of chickens at contact exposure on incidence of lymphomatosis. Poultry Sci. 28:254.

———, and Prickett, C. O.: 1944. The development of families free of lymphomatosis. Poultry Sci. 23:321.

Wickware, A. B.: 1943. Transmissible leucosis. A recently isolated strain. Canad. Jour. Comp. Med. 7:265.

———: 1946. The incidence of erythroleucosis following inoculation by various routes. Canad. Jour. Comp. Med 10.74.

Wight, P. A. L.: 1962a. Variations in peripheral nerve histopathology in fowl paralysis. Jour. Comp. Path. and Therap. 72:49.

———: 1962b. The histopathology of the central nervous system in fowl paralysis. Jour. Comp. Path. and Therap. 72.348.

———: 1963. Lymphoid leucosis and fowl paralysis in the quail. Vet. Record 75:685.

Wilcke, H. L., Patterson, P. D., Henderson, E. W., and Murray, C.: 1933. The effect of the ration upon the incidence of so called range paralysis. Poultry Sci. 12:226.

Winton, D., Lucas, E. H., and Cottral, G. E.: 1950. The effects of feeding tomatoes on the incidence of lymphomatosis in chickens. Poultry Sci. 29.912.

Zilber, L. A., and Kryukova, I. N.: 1957. Haemorrhagic disease of rats due to the virus of chick sarcoma. Acta Virologica (English ed.) 1:156.

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Electron Microscope Studies of Avian Leukosis Tumors*

Electron microscopy of ultrathin sections of chicken tumors has contributed greatly to our knowledge of the nature of the viruses causing these tumors and to our understanding of virus-cell relationship in the cells of tumors and leukemias of chickens. It has demonstrated the morphology of virus particles and the sites of their replication in the infected cells. Biological, biophysical, biochemical, as well as cytochemical studies combined with electron microscopy have contributed to significant advances in our knowledge of these agents and have led to a quantitative correlation between virus particles and the tumor-inducing properties of the tissues in which they have been observed. Electron microscopy has also helped to demonstrate that many cell types, as in the infectious processes induced by many nontumor viruses, may participate in the response of the susceptible host to an infection with avian tumor viruses.

ROUS SARCOMA

Previous studies on Rous sarcoma tumor cells and Rous sarcoma virus particles have been extensively presented in a number of reviews (Bernhard, 1958, 1960; Dmochowski, 1960a, b; Haguenau, 1960).

Particles suggestive of virus were first observed in Rous sarcoma cells grown in tissue culture (Claude *et al.*, 1947) before the introduction of the ultrathin sectioning technique to electron microscopy. It

took a number of years before virus particles were found in thin sections of this tumor (Gaylord, 1955). Subsequently, numerous studies on Rous tumor cells grown *in vivo* and *in vitro*, as well as on normal cells grown in tissue cultures infected with Rous sarcoma virus, confirmed and extended these early observations (Bernhard *et al.*, 1956; Haguenau *et al.*, 1958, 1960a, b; Haguenau, 1960).

Virus particles (800 Å) have been found at the cell surface and in vacuoles of the cytoplasm of tumor cells (Bernhard *et al.*, 1956) and in fibroblasts infected *in vitro* with Rous sarcoma virus (Haguenau *et al.*, 1960b). As seen in the electron microscope, the relationship of Rous sarcoma virus to the constituents of sarcoma cells may appear in a number of forms seen not only in the tumor cells grown *in vitro* or in the fibroblasts infected with the virus *in vitro* (Beard, 1963) but also in tumor cells grown *in vivo* (Dmochowski *et al.*, 1964).

Several types of cells, such as fibroblasts, macrophagelike cells, and mast cells have been described in the Rous sarcoma tumor (Haguenau and Beard, 1962). Cytoplasmic vacuoles, some containing characteristic virus particles, have been observed in all these cells (Haguenau and Beard, 1962; Dmochowski *et al.*, 1964). This is shown in Figures 19.25 and 19.26. No evidence of a specific relationship with virus particles could be seen in some of the vacuoles. The virus particles may be present in these vacuoles as the result of pinocytosis or phagocytosis, especially in the cells of the macrophage type (Haguenau and Beard,

[589]
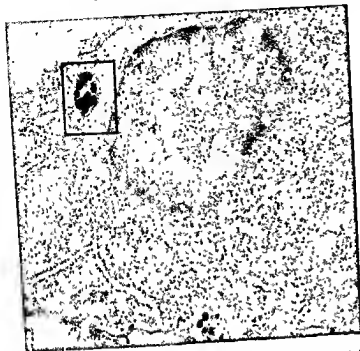
FIG. 19.25 — Appearance of a Rous sarcoma cell with virus particles adhering to the plasma membrane and with a cytoplasmic vacuole, containing virus particles (in frame). ×30,000.

1962). Structures have been found in Rous sarcoma cells grown *in vitro* (Haguenau and Beard, 1962) which consist of closely packed typical virus particles in a matrix of amorphous osmiophilic material, and apparently not surrounded by a limiting membrane. In our studies on Rous sarcoma induced by the Bryan strain of virus (Bryan, 1959), structures have been ob-

served in Rous sarcoma cells *in vivo* similar to those described in Rous sarcoma cells *in vitro*. In addition, structures have been found (Dmochowski *et al.*, 1964) identical in appearance to cytoplasmic inclusion bodies (Fig. 19.27) (Dmochowski *et al.*, 1961) or to gray bodies or viroplasts (Beard, 1968) observed in myeloblastosis, erythroblastosis, visceral lymphomatosis,

FIG. 19.26 — Part of Fig. 19.25 (in frame) at higher magnification. ×150,000.

FIG. 19.27 — Part of cytoplasm of Rous sarcoma cell, showing cytoplasmic inclusion, with virus particles, surrounded by a membrane. ×90,000.
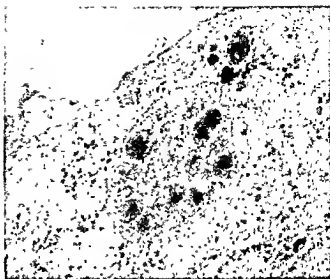
and nephroblastoma. What could be described as intermediate structures between those surrounded by a single or a double limiting membrane containing closely packed virus particles in a densely osmiophilie material and those without any evidence of a limiting membrane have also been found in Rous sarcoma cells *in vivo* (Dmochowski *et al.*, 1964). These structures appear to be loci of virus synthesis, and the disappearance of a membrane surrounding these structures may lead to virus

particle dissemination by a process the reverse of pinocytosis. The walled-in cytoplasmic inclusions have also been found to undergo progressive vacuolization like those in chicken leukosis which may be removed by a similar process of reverse pinocytosis.

Structures, similar to "viroplasm" (Beard, 1963), were originally described in the cytoplasm of Rous tumor cells by Bernhard *et al.* (1956). These structures, at first, could not be found in other studies

FIG. 19.28 — Part of cytoplasm of Rous sarcoma cell, showing viral matrix or viroplasm. ×30,000.
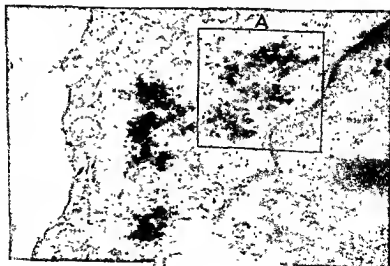
FIG. 19.28A — Part of the viral matrix, shown in Fig. 19.28 (in frame) at higher magnification. Arrows indicate virus particles in formation. ×83,000.

(Haguenau et al., 1958). The structures (Haguenau and Beard, 1962) consist of densely osmiophilic particles of the size of ribosomes and among them particles (650 A) composed of circular membranes and of a size smaller than the characteristic particles (800 A) usually encountered. An outer ring of ribonucleoprotein granules surrounds these particles (Haguenau and Beard, 1962). These structures appear in clusters in the cytoplasm of tumor cells (Dmochowski et al., 1964) (Figs. 19.28 and 19.28A). These small particles or granules described as virosomes (Heine et al., 1962b) and the circular images or virospheres may represent precursor material for the synthesis of Rous sarcoma virus (Haguenau and Beard, 1962). The virospheres may represent incomplete virus particles formed in an abortive process of synthesis (Beard, 1963). The structures described as viroplasm have been observed, as will be shown later, in myeloblastosis, erythroblastosis, visceral lymphomatosis, and nephroblastoma (Beard, 1963; Dmochowski et al., 1961).

It has recently been shown that Rous sarcoma virus is formed and liberated by budding of the plasma membrane of fibroblasts grown in vitro and infected with Rous virus (Haguenau et al., 1962) and

of the plasma membrane of tumor cells in vivo (Heine et al., 1962b; Dmochowski et al., 1964) (Figs. 19.29 and 19.30). This budding phenomenon has originally been described in erythroblastosis (Benedetti and Bernhard, 1958), in myeloblastosis (Dmochowski, 1961, 1963; Dmochowski et al., 1961), and in nephroblastoma (Dmochowski, 1961, 1963; Dmochowski et al., 1961). Budding of plasma membranes has recently been observed in visceral lymphomatosis (Dmochowski et al., 1961).

The budding of the plasma membrane

FIG. 19.29 — Budding of plasma membrane of a Rous sarcoma cell. ×180,000.
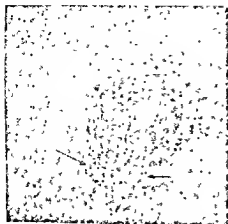
FIG. 19.30 — More advanced stage of budding. Arrows indicate the stalk with which an almost fully formed virus particle is attached to the plasma membrane. ×420,000.

of Rous sarcoma cells has not been noted in all the previous extensive studies (Bernhard et al., 1956; Epstein, 1957; Haguenau et al., 1958, 1960a, b). This may serve as an example of the error of sampling which may occur in electron microscope studies of any type of changes in cells and of the erroneous conclusions which may be based on such sampling. It appears that while the frequency of the changes of various types induced by chicken tumor viruses may vary, these viruses may all have a similar mode and similar sites of development with one or another site occurring more frequently, according to the type of infected cell, without any qualitative differences for any type of the virus strain involved.

Virus particles, similar to those found in vacuoles of the cytoplasm of Rous sarcoma cells and in the intercellular spaces of the Rous tumor are present in high-speed centrifugal pellets from extracts of Rous sarcoma tumors. A correlation could be established between the potency of tumor extracts and the number of virus particles seen in sections of these tumors or in the pellets from cell-free extracts of such tumors (Epstein, 1957, 1958; Haguenau et al., 1958).

No changes of a specific character could be observed in the nucleus or nucleolus of Rous sarcoma cells, except for small dense bodies present in the nuclear sap (Haguenau, 1960).

Studies on the ultrastructure of other chicken tumors such as Murray-Begg endothelioma (Rouiller et al., 1956) and of Fujinami myxosarcoma (Mannweiler and Bernhard, 1958) have led to the discovery of characteristic (800 A) virus particles, similar to those found in Rous sarcoma, but they have failed to demonstrate the changes observed in the studies on Rous sarcoma. These tumors require reinvestigation in the light of recent findings in Rous sarcoma.

MYELOBLASTOSIS (GRANULOBLASTOSIS)

The myeloblastosis strain of chicken leukosis, later described as the BAI strain A, was originally isolated by Hall et al. (1941) from chickens with neurolymphomatosis and later studied extensively by Johnson (1941) and by Beard and his associates (1956, 1963).

In sections of myeloblasts present in the spleen, bone marrow, and the circulating blood of chickens with myeloblastosis induced by BAI strain A myeloblastosis virus only few changes have been reported (Beard, 1963) which could be related to virus infection. These changes consisted of cytoplasmic vacuoles in myeloblasts, with occasional characteristic virus particles (800 A). While most of the virus particles were found outside the cells (Bonar et al., 1959, 1960; Parsons et al., 1959). Otherwise, the myeloblasts have been found similar to those present in normal chicken bone marrow (Beard, 1963). It is as yet unknown to what extent these findings have been associated with the stage of infection or of the progression of neoplasia. In other studies (Dmochowski, 1960 a–c, 1961, 1963; Dmochowski et al., 1958b, 1959a, 1961, 1964), examination of the spleen from chickens in advanced stages of myeloblastosis, particularly of the areas diagnosed histologically as replaced by neoplastic or tumorous cells (myeloblasts), changes have been observed in the myeloblasts or cells of the myeloid series which

could be interpreted as related to virus synthesis and comparable to those seen in myeloblasts grown *in vitro* and obtained from the infected chickens (Beard, 1963). Similar changes have been found in reticular cells and macrophages more frequently than in myeloblasts (Parsons *et al.*, 1959). While this is undoubtedly the case, there appears to be little doubt that in the advanced stages of myeloblastosis, changes in the cells present in the spleen which lead to the enlargement of the cytoplasm of the cells, make the diagnosis of the type of cell involved frequently difficult. However, similar changes have been observed in the reticular cells, macrophages, and myeloblasts in the spleen of chickens with myeloblastosis (Dmochowski *et al.*, 1964).

The changes consist of cytoplasmic inclusions (Dmochowski, 1961) or gray bodies or viroplasts (Beard, 1963) in various stages of formation and vacuolization, fully or partially filled with the characteristic virus particles (800 A). These inclusions, surrounded by a single or a double membrane or apparently without a membrane (Figs. 19.31, 19.32, 19.32A, 19.33, 19.33A, 19.33B), filled with osmiophilic material of varying density, could conceivably be interpreted as related to mitochondria. They frequently

appear to be in various stages of vacuolization and contain virus particles in varying numbers.

Less frequently encountered changes consist of aggregations of dense osmiophilic granules (Figs. 19.34 and 19.34A with apparently empty circular images, similar in many respects to the structures present in the cytoplasm of Rous sarcoma cells and in that of erythroblasts (Beard, 1963; Dmochowski *et al.*, 1964), and in the epithelial cells of nephroblastoma (Dmochowski *et al.*, 1961).

Further changes, observed in myeloblasts in the spleen of chickens with myeloblastosis, also less frequently encountered than the cytoplasmic inclusions or gray bodies or viroplasts, consist of the budding of plasma membrane of myeloblasts which apparently leads to virus particle formation (Dmochowski *et al.*, 1961, 1964; Beard, 1963) (see Fig. 19.35).

Similar changes, but more frequently present, have been observed in myeloblasts grown *in vitro* (Parsons *et al.*, 1958; Bonar *et al.*, 1959, 1960). Cytoplasmic inclusions (gray bodies or viroplasts) of varying number and size, and with virus particles in various stages of formation, have been found to increase in frequency and extent
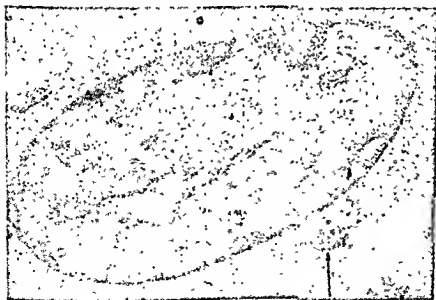
FIG. 19.31—Appearance of a cell in the spleen of chicken with myeloblastosis with an inclusion containing virus particles present in the cytoplasm. ×28,000.

FIG. 19.32 — Appearance of another cell in the spleen of a chicken with myeloblastosis, showing numerous cytoplasmic inclusions (A) containing virus particles. ×12,000.

with the time of growth of myeloblasts *in vitro* (Bonar et al., 1960). These inclusions change in appearance, alter in density, and undergo vacuolization. These structures appear to be the sites of virus formation (Parsons et al., 1958). No particular localization of these bodies in the cytoplasm could be noted. The presence of virus particles at all stages of the changes in the viroplasts indicates that these structures are involved in virus synthesis (Haguenau and Beard, 1962). It is likely that the substance of cytoplasmic inclusions or viroplasts is used for virus particle formation which then may be transported in the vacuoles to the cell surface (Beard, 1963). These structures appear to be specific loci of virus synthesis as, like the coat of virus particles, they dephosphorylate adenosine triphosphate (Beaudreau et al., 1958). They are similar in size to mitochondria.

There is conflicting evidence available about the origin of these cytoplasmic inclusions or viroplasts. Although no virus particles have been found in unaltered mitochondria (Bonar et al., 1960), an anal-

FIG. 19.32A — Part of the cell in Fig. 19.32, marked A, showing cytoplasmic inclusions with virus particles in different stages of formation. ×60,000.
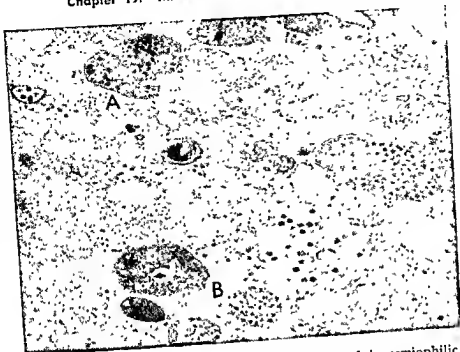
FIG. 19.33 — Part of the cytoplasm of a macrophage in the spleen of a chicken with myeloblastosis, showing cytoplasmic inclusions, some marked A and B, in different stages of vacuolization, containing virus particles. ×23,000.

ysis of the appearance of the various cytoplasmic inclusions appears to indicate that mitochondria undergo a series of alterations, starting from the loss of internal structure which is being gradually replaced by dense osmiophilic material in which virus particles appear (Dmochowski et al., 1961, 1964). The increase in the number of particles is associated with the disappearance of the osmiophilic material and is followed by progressive vacuolization of the cytoplasmic inclusions. In such inclusions, strands (Bonar et al., 1959, 1960) and membranes (Dmochowski and Grey, 1958; Dmochowski et al., 1958b, 1959a, 1961; Dmochowski, 1960 a–c, 1961) have been observed. These membranes or strands may, but need not necessarily, indicate the origin

FIG. 19.33A — Part of the cell in Fig. 19.33, marked A, at higher magnification. ×64,000.
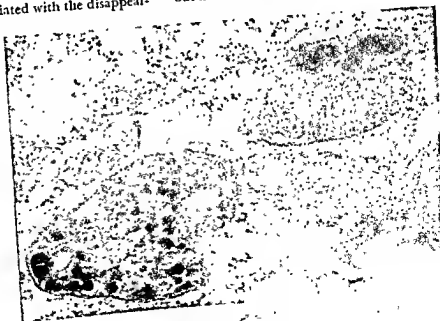
FIG. 19.33B—Part of the cell in Fig. 19.33, marked B, at higher magnification. ×64,000.

of the cytoplasmic inclusions from altered mitochondria.

There is substantial evidence available that the viroplasts do not develop from mitochondria but from the granules or their precursors which appear in the cells of myeloid series during their normal maturation to the granulocytic series of blood cells (Haddad et al., 1960; Weinstein et al., 1960; Sommer et al., 1962). This evidence is based on cytochemical studies which demonstrated adenosine triphosphatase activity in these granules, in viroplasts or cytoplasmic inclusions, and in the outer coat of virus particles (Beard et al., 1963). These studies have also led to the conclu-

sion that the granules develop from precursor material in the cytoplasm (Beard, 1963) and not, as originally suggested, from mitochondria (Bernhard et al., 1955). Viroplasts as well as the granules of normal myelocytes exhibit strong adenosine triphosphatase activity. There appears to be however a considerable difference between the enzymes per se of the normal granules and those of the viroplasts, as shown by freeze-drying in vacuo and by fixation with formaldehyde (Weinstein et al., 1960). The size and the number of viroplasts increase in cultures containing 5-methyl tryptophan (Weinstein et al., 1960). Myeloblasts taken

FIG. 19.34—Part of the cytoplasm of a cell in the spleen of chicken with myeloblastosis, showing viral matrix or viroplasm, framed and marked with letter A. ×23,000.
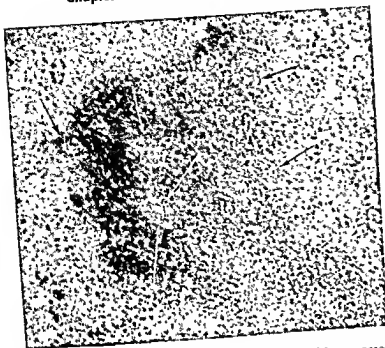
FIG. 1934A—Part of Fig. 19.34 at higher magnification. Arrows indicate circular images surrounded by dense osmiophilic granules. X77,000.

directly from the circulating blood of chickens with myeloblastosis only seldom show the presence of viroplasts and little or no evidence of adenosine triphosphatase activity (Haddad *et al.*, 1960). The results of phase contrast microscopy and of staining for adenosine triphosphatase parallel the observations made in the electron microscope (Haddad *et al.*, 1960).

Studies of the bone marrow of normal chickens grown in tissue culture and infected with myeloblastosis virus (Beaudreau *et al.*, 1958; 1960) have demonstrated that after 3 weeks in culture, the infected cells show multiplication of myeloblasts with liberation of myeloblastosis virus in tissue culture fluids. The ultrastructure of these cells is identical with that of myeloblasts obtained from the circulating blood of chickens with myeloblastosis and grown *in vitro*. Myeloblasts from both sources show structures in the cytoplasm, the so-called viroplasts or cytoplasmic inclusions. Both types of cells reproduce myeloblastosis in chickens (Beaudreau *et al.*, 1960).

Thus, tissue culture studies of myeloblasts grown *in vitro* confirmed and extended (Haguenau and Beard, 1962; Beard, 1963) the observations reported on myeloblasts present in the spleen, liver, and bone marrow of chickens with myeloblastosis. However, no structures such as viroplasm or viral matrix have as yet been observed in myeloblasts maintained in tissue culture (Beard, 1963).

The formation of myeloblastosis virus in cytoplasmic inclusions or viroplasts of myeloblasts and in the viral matrix or viroplasm apparently are not the only mode of virus synthesis. Budding of the plasma membrane of myeloblastosis has been shown to be another way of myeloblastosis virus formation (Dmochowski, 1961; 1963; Dmochowski *et al.*, 1961, 1964). Recently in isolated instances, it has also been observed by Beard (1963). There is as yet no evidence of any connection between the budding of plasma membrane and virus formation in cytoplasmic inclusions or in viral matrix.

ERYTHROBLASTOSIS

Numerous electron microscope studies have been carried out on organs and tissues, such as spleen, liver, and bone marrow, of chickens with erythroblastosis induced by the virus of different strains of origin (Benedetti and Leplus, 1958;

FIG. 19 35—Budding of plasma membrane (arrow) of a myeloblast in the spleen of a chicken with myeloblastosis. ×90,000.
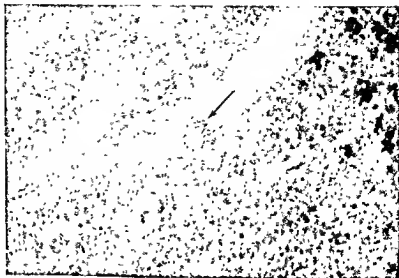
Benedetti and Bernhard, 1958; Iwakata and Amano, 1958; Iwakata, 1958), including strain R of Engelbreth-Holm and Rothe-Meyer (1935) (Dmochowski et al., 1958a, 1959a; Dmochowski, 1960a–c, 1961, 1963; Heine et al., 1961) and strain RPL-12 (Dmochowski et al., 1959b) The results of all these studies have been similar, although interpretation of the findings has differed. Studies have also been carried out on erythroblasts grown in tissue culture

and on those present in the circulating blood of chickens with erythroblastosis induced by strain R (Heine et al., 1961).

In some studies (Beard, 1963), few changes have been observed in the erythroblasts present in the spleen and bone marrow of leukemic chickens, except for occasional virus particles in vacuoles of the cytoplasm of erythroblasts. Greater changes such as vacuolization of the cytoplasm with virus particles present in these vacuoles,

FIG. 19.36— Part of the cytoplasm of a cell in the spleen of a chicken with erythroblastosis showing cytoplasmic inclusions in various stages of vacuolization, containing virus particles. ×15,000.

FIG. 19.37—Port of the cytoplasm of another cell in the spleen of a chicken with erythroblastosis showing vacuoles framed and marked A, containing virus particles. ×23,000.

and inside the cytoplasm of cells as well as budding of plasma membranes leading to the formation of virus particles have been observed in the circulating erythroblasts and in those grown in tissue culture (Heine et al., 1961). Characteristic virus particles (800 A) have also been observed in tissue culture fluids by electron microscopy and by biological tests (Heine et al., 1961). In addition, in a few cases a structure, the so-called viroplasm or viral matrix, has been observed in the erythroblasts grown in vitro. As already mentioned in the discussion on Rous sarcoma and myeloblastosis, this is a change in the cytoplasm, composed of particles of the size of ribosomes, but of greater electron density (Heine et al., 1961). Within this viroplasm or viral matrix particles have been found resembling the incomplete particles present in the cytoplasm of Rous sarcoma cells (Haguenau and Beard, 1962) and in nephroblastoma cells (Dmochowski, 1961, 1963; Dmochowski et al., 1961; Heine et al., 1962a). No evidence of adenosine triphosphatase activity has been observed in the
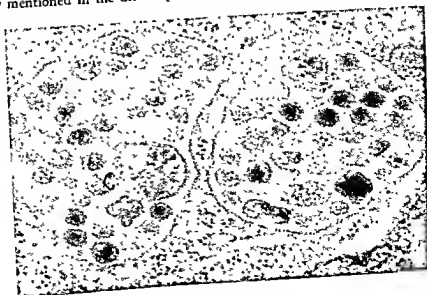
FIG. 19.37A—Part of Fig. 19.37 at higher magnification. Variation in the size of virus particles may be seen. ×90,000.

FIG. 19.38 — Low magnifica-
tion view of another cell in
the spleen of a chicken with
erythroblastosis, showing cy-
toplasmic inclusions, marked
A. ×23,000.

erythroblasts from the bone marrow of
normal chickens or those with erythro-
blastosis (Bonar et al., 1957; Beard, 1963).

Erythroblasts and cells of the erythroid
series, like myeloblasts and cells of the
myeloid series, present in the spleen and
liver of chickens with erythroblastosis
strain R, showing neoplastic involvement,
have been found to show changes similar

to those observed in myeloblasts (Dmo-
chowski, 1961, 1963). However, these
changes as in myeloblasts are not as fre-
quently encountered in the erythroblasts
as in the reticulo-endothelial cells or in
macrophages present in the spleen of chick-
ens with erythroblastosis (Benedetti and
Bernhard, 1958; Benedetti and Leplus,
1958; Dmochowski et al., 1958a, 1959a, b,

FIG. 19.38A — Part
of Fig. 19.38 at
higher magnifica-
tion showing cyto-
plasmic inclusions
in different stages
of vacuolization
with virus particles.
×46,000.

FIG. 19.39 — Cytoplasmic inclusion in erythroblastosis, showing in parts a double membrane, containing virus particles. ×90,000.

1961; Dmochowski, 1960a, c; Heine et al., 1961; Iwakata, 1958; Iwakata and Amano, 1958). Characteristic virus particles (800 A) have been found in the intercellular spaces and in cytoplasmic vacuoles with varying amounts of osmiophilic material (Figs.

19.36, 19.37, 19.37A), as well as in cytoplasmic inclusions of the size of mitochondria or larger (Figs. 19.38, 19.38A, 19.39). In addition, whorl-like formations have been observed in the erythroblasts. Occasionally, virus particles have been observed
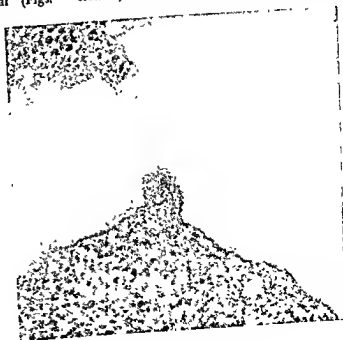
FIG. 19.40 — Budding of plasma membrane of an erythroblast in the spleen of chicken with erythroblastosis. ×120,000.

FIG. 19.41 — Viral matrix or viroplasm (A) in the cytoplasm of an erythroblast, circular images in matrix marked by arrows. ×30,000.

to form by budding of plasma membrane (Fig. 19.40) of erythroblasts (Dmochowski et al., 1961, 1964). Equally rare are the structures, similar to viroplasm or viral matrix in the cytoplasm of myeloblasts, with circular images surrounded by dense osmiophilic particles which have been ob-

served in the cytoplasm of erythroblasts (Figs. 19.41 and 19.41A) present in the spleen of chickens with erythroblastosis strain R (Beard, 1963; Dmochowski et al., 1964).

Occasionally, structures have been observed with dense osmiophilic granules of

FIG. 19.41A — Part of Fig. 19.41 at higher magnification. Circular images (arrows) surrounded by dense osmiophilic granules. ×68,000.

FIG. 19.42 — Part of another cell in the spleen of chicken with erythroblastosis showing densely osmiophilic viral matrix (A). ×23,000.

FIG. 19.42A — Part of Fig. 19.42 at higher magnification showing fully formed typical virus particles in the viral matrix. ×68,000.

the size of ribosomes and with virus particles, fully formed or almost fully formed, lying on the periphery of the aggregates of the granules (Figs. 19.42 and 19.42A). It is quite possible that these structures constitute a further stage in the synthesis of erythroblastosis virus particles (Dmochowski et al., 1964). In addition, occasionally structures have been observed apparently in the nuclei of erythroblasts in the spleen of the infected chickens which give internal images forming among what at first sight appeared to be structures identical to viral matrix or viroplasm (Fig. 19.43). On close inspection Dmochowski et al. (1964) observed that these structures are composed not of granules but of strands, similar to those observed in the nuclei of other erythroblasts, and among them are circular images similar to those in the cytoplasmic viral matrix or viroplasm (Figs. 19.42 and 19.42A). They may be nuclear pores.

No indication of nuclear involvement has as yet been detected in Rous sarcoma tumor cells or in any cells of organs or tissues of chickens with avian leukosis except for hypertrophy of the nucleolus and small dense bodies in the nuclear sap

(Haguenau and Beard, 1962; Beard, 1963). Nevertheless, Rous sarcoma antigen has been found in the nucleus of virus-infected cells by means of labeled specific antibody before the appearance of the antigen in the cytoplasm of these cells (Malmgren et al., 1960; Mellors, 1960; Noyes, 1960). The connection between the immunological findings in Rous sarcoma cells and the electron microscope findings in erythroblastosis of chickens infected with erythroblastosis strain R virus is as yet unknown, but it may well constitute the first electron microscope observation of chicken tumor virus formation in the nucleus if the presence of Gallus adenolike virus of chickens (Burmester et al., 1960d), is excluded.

The cytoplasmic structures may be loci of virus synthesis, although the cytoplasmic inclusions have also been interpreted as the result of phagocytosis (Haguenau and Beard, 1962; Beard, 1963). This matter still remains to be resolved.

The described changes could not be as clearly and as frequently visualized in erythroblasts grown in vitro (Heine et al., 1961) as in myeloblasts grown in tissue culture (Bonar et al., 1960). The presence of

FIG. 19.43 — Nucleus of a cell in the spleen of a chicken with erythroblastosis. Arrows indicate circular images, similar to those found in cytoplasmic viral matrix in Figs. 17.41 and 19.41A. ×30,000.

numerous virus particles in the cytoplasmic vacuoles of erythroblasts grown *in vitro* was interpreted as the result of phagocytosis (Heine *et al.*, 1961). The budding phenomenon of the plasma membrane of erythroblasts grown *in vitro* has also been observed as in the case of erythroblasts grown in tissue culture (Heine *et al.*, 1961). While tissue culture has undoubtedly helped the electron microscope studies on Rous sarcoma and on myeloblastosis, it has been of particular help in the quantitative studies on these tumor viruses. Its great value is based on compelling investigators to re-examine the previously reported findings of electron microscope studies of Rous sarcoma and of leukemias of chickens. This re-evaluation of electron microscope examination has revealed changes in Rous sarcoma cells and in tissues of chickens with myeloblastosis and erythroblastosis, previously unobserved, and identical with those found in the respective tumor cells grown *in vitro*.

Virus particles and cytoplasmic inclusions have also been observed in macrophages and in reticular cells present in various tissues of chickens with erythroblastosis (Benedetti and Bernhard, 1958; Benedetti and Leplus, 1958; Dmochowski *et al.*, 1961). In these studies, cytoplasmic inclusions or viroplasts or gray bodies have been found more frequently than in the tumorous cells or erythroblasts. An analysis of the various changes observed in the cytoplasmic inclusions of reticular cells or of macrophages, as in the case of erythroblasts, indicates that they may be of mitochondrial origin, as originally suggested by Benedetti and Bernhard (1958). However, no budding of plasma membrane and no viroplasm (viral matrix) could be found in the reticular cells and macrophages. This does not necessarily indicate that such changes may not take place in these cells.

Alterations and changes observed in the erythroblasts or tumorous cells, reticular cells, and in macrophages present in the spleen and liver of chickens with erythroblastosis strain R (Dmochowski *et al.*,

1958a) are similar to those observed in the cells of the same origin present in the spleen and liver of chickens with erythroblastosis induced by strain RPL-12 (Dmochowski, 1960a-c, 1961, 1963; Dmochowski *et al.*, 1959b, 1961). The difference in the frequency of changes observed in the erythroblasts present in various organs and tissues of chickens with erythroblastosis, observed by different investigators may, at least in part, be due to the stage of disease at which these organs have been examined and to the extent of infiltration of the various organs by erythroblasts or tumorous cells.

Comment

The changes seen in myeloblastosis and in erythroblastosis have been described as of two types: nonspecific and specific with regard to pathological changes and to evidence of the relationship of the respective viruses to primary neoplastic cells (Haguenau and Beard, 1962).

As one of the nonspecific changes, the presence of characteristic virus particles has been considered in reticular cells and in macrophages in the spleen, liver, and bone marrow of chickens with myeloblastosis or erythroblastosis. Similarly, the presence of cytoplasmic inclusions containing virus particles in the cells of the spleen, liver, or bone marrow of chickens with myeloor erythroblastosis and with visceral lymphomatosis has been interpreted as a nonspecific change and attributed to phagocytosis (Haguenau and Beard, 1962). In other studies, macrophages have been considered as reservoirs of chicken tumor viruses and as sites of their intercellular proliferation (Iwakata, 1958; Iwakata and Amano, 1958). Whether the observed changes in reticular cells and macrophages can be attributed solely to phagocytosis of the respective viruses remains to be determined. In view of the presence of changes which can be interpreted as loci of virus synthesis in cells which do not participate directly in the neoplastic process such as those of the pancreas, thymus gland, and liver of chickens infected with

FIG. 19.44 — Low-power view of a cell in the spleen of a chicken with visceral lymphomatosis showing an inclusion with virus particles. ×15,000.

croscope, in response to viruses of myeloblastosis, erythroblastosis, and Rous sarcoma, and later found to be either nonexistent or only quantitative, are indeed quantitative or qualitative. Such studies may help to resolve the problem whether one or a number of viruses are involved in the induction of chicken leukosis and other tumors of birds.

LYMPHOMATOSIS

The lymphomatosis strain RPL-12 virus was isolated (Burmester, 1947) from a lymphosarcoma originally described by Olson (1941). The RPL-12 virus has been found to induce, besides "extravascular" visceral lymphomatosis and osteopetrosis, the so-called "intravascular" visceral lym-
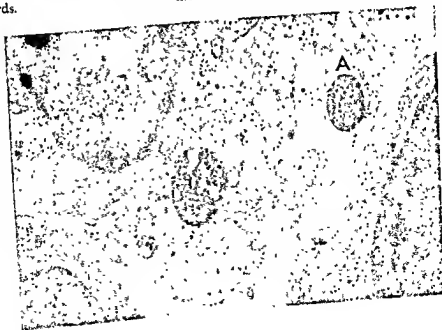
FIG. 19.45 — Part of the cytoplasm of a lymphoblast, showing cytoplasmic inclusions, one marked A. ×23,000.

FIG. 19.44 — Low-power view of a cell in the spleen of a chicken with visceral lymphomatosis showing an inclusion with virus particles. ×15,000.

croscope, in response to viruses of myelo-blastosis, erythroblastosis and Rous sarcoma, and later found to be either nonexistent or only quantitative, are indeed quantitative or qualitative. Such studies may help to resolve the question whether one or a number of viruses are involved in the induction of chicken leukosis and other tumors of birds.

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FIG. 19.45 — Part of the cytoplasm of a lymphocyte showing cytoplasmic inclusion one micron in diameter.

FIG. 19.45 A – Part of Fig. 19.45 at higher magnification showing cytoplasmic inclusion, without a membrane, containing virus particles, ×90,000.

phomatosis, later reclassified as erythroblastosis (Burmester et al., 1959a, 1960a, c; Gross et al., 1959). The virus of visceral lymphomatosis, obtained from both natural and experimentally induced disease, as well as from livers of embryos of apparently normal birds, induces not only visceral lymphomatosis but erythroblastosis (Burmester et al., 1959a). Occasionally it also causes osteopetrosis, hemangiomas, fibrosarcomas, myxosarcomas, myelocytomas, neurolymphomatosis, ocular lymphomatosis, and granuloblastosis (myeloblastosis). Chickens, reared in contact with birds inoculated with strain RPL-12 lymphomatosis, have been observed to develop occasionally erythroblastosis or "intravascular" visceral lymphomatosis (Burmester et al., 1959a). As shown by Burmester et al. (1959b), "extravascular" visceral lymphomatosis which arises intra- or extramedullarly in which the cell type is a highly undifferentiated element of the lymphoid series (Gross et al., 1959) and hence described as tumorous (Dmochowski, 1960a, b, c; 1961; 1963) is also induced by strain R of erythroblastosis (originally described by Engelbreth-Holm and Rothe-Meyer, 1932). Pathologically, the "extravascular"

visceral lymphomatosis induced by RPL-12 strain does not differ from that induced by strain R, or from that induced by myeloblastosis BAI strain A virus (Burmester et al., 1959b). These observations may have some bearing on the results of electron microscope studies of organs and tissues from chickens with visceral or "extravascular" lymphomatosis.

Examination of the parts of spleen of chickens with visceral lymphomatosis showing neoplastic involvement revealed certain characteristic changes in cells, such as reticulo-endothelial cells, macrophages and lymphoblasts (tumorous cells), or cells of the lymphoid series (Dmochowski, 1960a, b, c; 1961; 1963; Dmochowski and Grey, 1958; Dmochowski et al., 1959a, c; 1964). In addition, virus particles have been found in the intercellular spaces. Alterations in mitochondria and endoplasmic reticulum have been observed, with nucleus and nucleolus, showing changes such as alteration in size and density, which need not necessarily indicate a specific effect of viral infection. Cytoplasmic inclusions of the size of mitochondria or larger, filled with dense osmiophilic material undergoing various stages of vacuolization with

FIG. 19.46 — Part of the cytoplasm of a cell present in the spleen of a chicken with visceral lymphomatosis. Numerous cytoplasmic inclusions (A) in various stages of formation, some with virus particles. ×15,000.
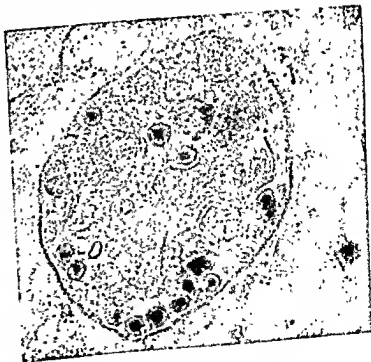
FIG. 19.46A — Part of Figure 19.46 showing a cytoplasmic inclusion with membranous structures and virus particles. ×75,000.

FIG. 19.47—Budding of plasma membrane of two lymphoblasts. ×90,000.

virus particles present in varying numbers, have been observed in all three types of cells apparently with least frequency in lymphoid cells (Fig. 19.44, 19.45, 19.45A, 19.46, 19.46A). In addition, occasional "budding" of plasma membrane (Fig. 19.47) and very occasionally structures composed of the formation of dense small osmiophilic particles with circular images

or viroplasm, similar to those present in Rous sarcoma, myeloblastosis, and erythroblastosis, have been found in the cytoplasm of cells which could only be described as cells of the lymphoid series. In addition, "whorl-like" formations have been observed in these cells as well as in the reticulo-endothelial cells and in macrophages.

FIG. 19.48—Low power view of cells in nephroblastoma. Budding of plasma membranes (arrows) and intercellular virus particles. ×45,000.

FIG. 19.49 — Cytoplasmic inclusions with virus particles in a cell of nephroblastoma. ×30,000.

The changes have been observed in the cells of spleen from chickens with visceral lymphomatosis, whether induced by strain RPL-12 or strain R virus (Dmochowski et al., 1964). The findings confirm and extend the original observations made on cells in the spleen from chickens with RPL-12 strain induced lymphomatosis (Dmochowski et al., 1959c; Dmochowski, 1960a-c, 1961; 1963).

NEPHROBLASTOMA

Burmester et al. (1959b) and Thorell (1958, 1960) demonstrated that myeloblastosis BAI-A strain virus induces myeloblastosis, visceral lymphomatosis, osteopetrosis, and renal adenocarcinoma, later described by Burmester as nephroblastoma. This original observation has been confirmed by Baluda and Jamieson (1961) and by Ishiguro et al. (1962).

FIG. 19.50 — Higher view of cytoplasmic inclusions in nephroblastoma, partly vacuolized and containing virus particles. ×68,000

FIG. 19.47 – Budding of plasma membrane of two lymphoblasts. ×90,000.

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FIG. 19.50 — Higher view of cytoplasmic inclusions in nephroblastoma, partly vacuolized and containing virus particles. ×68,000.

FIG. 19.51 — Viral matrix or virus sum and budding particle in nephroblastoma cell. ×30,000.

FIG. 19.52 — High magnification of part of viral matrix showing circular images (arrows) and dense osmiophilic granules. ×117,00.

An electron microscope study (Dmochowski et al., 1960, 1961, 1964; Dmochowski, 1961, 1963) of nephroblastoma, both induced and transplanted, has revealed numerous intercellular and occasional cytoplasmic virus particles (Fig. 19.48). Cytoplasmic inclusions, similar to viroplasts in myeloblastosis (Bonar et al., 1960; Haddad et al., 1960; Weinstein et al., 1960; Dmochowski et al., 1958b; 1959a), filled with dense osmiophilic material, undergoing various stages of vacuolization with virus particles, either few or numerous, have been observed (Figs. 19.49 and 19.50). Budding of plasma membrane of cells leading to virus particle formation (Dmochowski et al., 1961) has been found more frequently (Dmochowski et al., 1964) than in erythroblastosis, myeloblastosis, or lymphomatosis or in Rous sarcoma cells (Figs. 19.48 and 19.51). Aggregates of dense osmiophilic particles of the size of ribosomes described as viral matrix, with various stages of virus particle formation more numerous than in other chicken neoplasias have been observed (Figs. 19.51 and 19.52). Frequently, cells have been found showing simultaneously the budding of plasma membrane, viral matrix, and cytoplasmic inclusions. If these changes are judged as sites of virus synthesis in cells of nephroblastoma, they have been found more numerous than in the cells of any other chicken neoplasia so far examined (Dmochowski et al., 1961, 1964).

These observations pertaining to the epithelial elements of nephroblastoma have been confirmed and extended to other cellular elements of this tumor (Heine et al., 1962a). Only occasional budding of plasma membrane but no other symptoms of viral synthesis have been found in the stromal cells, sarcomatous cells, and chondrocytes.

In these studies (Haguenau and Beard, 1962) no relationship could be established between the viroplasm or viral matrix and the formation of virus particles by nephroblastoma cells. In other studies (Dmochowski et al., 1961) formation or assembly of virus particles has been observed within the viroplasm or viral matrix in cells showing the formation of virus particles by plasma membranes, although this does not necessarily indicate a relationship between these two sites of virus formation.

The characteristic virus particles, while of the average size of 800 Å in cytoplasmic inclusions and in viral matrix or viroplasm, have been found to vary considerably in size, up to 1400 Å in diameter, when found in the intercellular spaces.

Adenosine triphosphatase activity was observed in nephroblastoma along the plasma membrane of cells of proximal and distal convoluted tubules and in the budding virus particles from these cells (de Thé et al., 1963c). However, sarcoma and cartilage plasma cell membranes and those of glomerular cells do not show the enzyme; virus particles which develop from these cells also do not show the enzyme (de Thé et al., 1963c). Virus particles from blood plasma of chickens with myeloblastosis show enzyme activity and are infective (Beard, 1963). It would be of interest to find out if virus particles can be obtained from nephroblastoma which do not show enzyme activity and are infective.

NONNEOPLASTIC CELLS AND TISSUES

As in other infections with the so-called ordinary or infectious viruses, cells and tissues which do not participate directly in the neoplastic process have been found infected with chicken tumor viruses. This is an important observation and may have an important bearing on the interpretation of the changes observed in reticular cells and in macrophages present in the spleen of chickens with erythroblastosis, myeloblastosis, visceral lymphomatosis, and also Rous sarcoma.

Thymus Gland

A study of the thymus gland of chickens with myeloblastosis (de Thé et al., 1963a) has demonstrated replacement of the lymphocytes of the cortex but not of the medulla by blast cells with the presence in the cytoplasm of these cells of spherical particles (650–850 Å) surrounded by a thin,

FIG. 19.51 — Viral matrix or viraplasm and budding (arrow) in nephroblastoma cell. ×30,000.
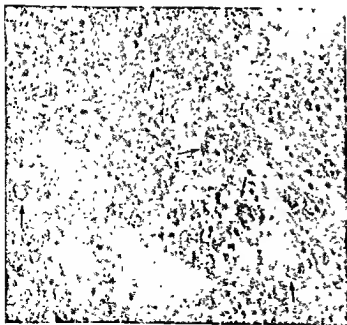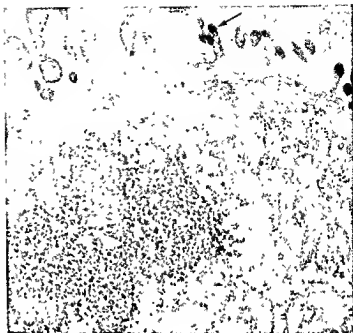
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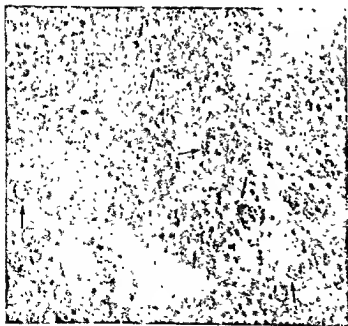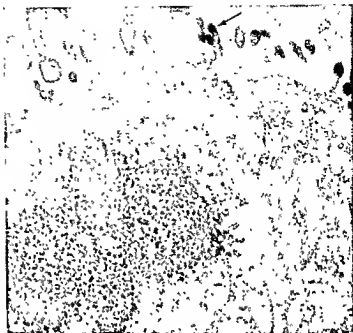
FIG. 19.52 — High magnification of part of viral matrix showing circular images (arrows) and dense osmiophilic granules. ×117,00.

dense ring, and enclosed by a less dense structure of 100–120 Å in thickness. Occasional budding of plasma membranes of the blast cells leading to virus particle formation has also been observed. Cytochemical studies have revealed the presence of adenosine triphosphatase activity of the plasma membranes and on the surface of individual virus particles. Similar studies of the thymus gland of chickens with erythroblastosis strain R have failed to reveal any specific changes. The blast cells may be a response of the reticular or lymphoid cells to BAI strain A virus (de Thé et al., 1963a). Cells of chickens with nephroblastoma which produce the same virus by "budding" apparently do not show these cytoplasmic changes (Heine et al., 1962a)

The enzyme adenosine triphosphatase first demonstrated in viroplasts or cytoplasmic inclusions of myeloblasts (Bonar et al., 1960) has also been found in the budding plasma membrane of the blast cells present in the thymus gland (de Thé et al., 1963a). It is also present, as already mentioned, in the plasma membrane of some epithelial cells of nephroblastoma and in their budding (Heine et al., 1962a). It is of interest that other cells of nephroblastoma apparently do not exhibit the same enzyme activity, although they appear to form virus by budding of their plasma membrane (de Thé et al., 1962).

Virus particles have also been found in the intercellular spaces of the thymus gland and in cytoplasmic vacuoles of cells described as of reticular origin.

Pancreas

Acinar cells of the pancreas of chicken embryos from supposedly normal hens and of apparently healthy chicks have been found to show budding of plasma membrane (Zeigel, 1961) similar to that observed in lymphoblasts, myeloblasts, erythroblasts, and in the epithelial cells of nephroblastoma (Dmochowski et al., 1960, 1961). The occurrence of the budding phenomenon has been found very frequently in the cells of the pancreas. It has not been found in the cells of the liver,

lung, kidney, spleen, small intestine, and heart, although characteristic virus particles (800 Å) composed of two concentric double membranes and a central dense nucleoid, have been observed in the intercellular spaces, in the cytoplasmic vesicles, and in vacuoles of cells, in all these organs as well as in the pancreas. The cells of the pancreas may be a site of tumor virus formation in latent or subclinical infections (Zeigel, 1961).

As yet there is no evidence that the characteristic virus particles in the pancreas of normal chickens are infectious particles, although their structure is identical with that of the particles found in Rous sarcoma, erythroblastosis, myeloblastosis, and visceral lymphomatosis ("extravascular"). This similarity gains in significance in view of the results of studies on the transmission of avian tumor viruses, such as visceral lymphomatosis (Burmester, 1957), Rous sarcoma, and erythroblastosis-lymphomatosis (Burmester et al., 1960a, b, c).

Characteristic virus particles in the spleen and in the bone marrow of normal chickens and apparently normal embryos have been described previously (Benedetti, 1957; Benedetti et al., 1956). They have also been described in tissue culture of such cells (Febvre and Benedetti, 1958). They have been found extracellularly in cytoplasmic vacuoles, or vesicles, and in cytoplasmic inclusions or viroplasm. Some of the structures in the different cells may be the result of phagocytosis, but at least some are due to the infectious process.

Similar evidence of the presence of virus in chick embryos has been obtained in another way (Rubin, 1960). An agent was found in cultures of tissues from some embryos which inhibited the infection of cells by Rous sarcoma. This agent was found to be the RPL-12 strain virus. Further extracts of apparently normal chick embryos have been found to transmit the neoplastic disease (Burmester, 1952). The recently reported RIF (resistance inducing factor) (Rubin, 1961) may be latent lymphomatosis virus, known to be present in many

matosis (Dmochowski *et al.*, 1959c). These cells appear to participate in virus synthesis more actively and more frequently than the respective tumorous cells of the erythroid, myeloid, or lymphoid series, but not exclusively, as reported by Beard (1963). Macrophagelike cells in Rous sarcoma cells grown *in vivo* or *in vitro* show changes (Beard, 1963) similar to those in macrophages studied in all forms of chicken leukosis. There is no need to accept the contention that macrophages or reticular cells have neoplastic traits, when concluding that these cells may also serve as seats of virus formation. It has indeed been proposed that macrophages may be the site of maturation of virus liberated in incomplete form by other cells in erythroblastosis (Iwakata and Amano, 1958).

The macrophages in the spleen of infected chickens are frequently seen in various stages of degeneration while there appears to be no lethal effect on myeloblasts grown *in vitro* (Beard, 1963). However, a comparison of the appearance of cytoplasmic inclusions or viroplasts in myeloblasts grown *in vitro*, as shown by Bonar *et al.* (1959, 1960) or in erythroblasts grown *in vitro* (Heine *et al.*, 1961) with that of cytoplasmic inclusions found in macrophages in the spleen of chickens with these diseases has failed to reveal any differences. Thus it appears that viral synthesis may take place in macrophages, while there is no doubt that some virus particles are phagocytized. If it is accepted that all cytoplasmic inclusions, contrary to morphological evidence, are phagocytized, the question of their origin arises. If macrophages do not synthesize the virus, then such inclusions must come from myeloblasts, erythroblasts, or lymphoblasts which would indicate that such cells also may degenerate in the spleen or liver of the affected chickens. The blast cells found in the spleen or liver of the diseased tissues have been designated "tumorous" (Dmochowski, 1960a–c, 1961, 1963; Dmochowski *et al.*, 1958a, b; 1959a, b, c) and not the reticular cells or macrophages (Beard, 1963). In these studies the term "tumorous" was used in preference to a specific designation such as myeloblasts or erythroblasts in view of the involvement of the myeloid or erythroid series of blood elements and therefore constituted a broader description of the specific types of cells encountered in the organs of the infected chickens.

PROPERTIES OF THE VIRUSES

An impressive amount of data is now available on the physical, chemical, and biological properties of the viruses involved in chicken neoplasia (Beard, 1963; Beard *et al.*, 1963). Considerable information has also been obtained on Rous sarcoma virus by the application of density gradient sedimentation (Crawford, 1960; Crawford and Crawford, 1961). There is no doubt that the virus particles observed in the neoplastic tissues or in the blood plasma of diseased chickens are the agents responsible for the various types of chicken neoplasia. Greater details of the evidence available in this respect are presented in a recent review by Beard (1963).

Particles (600–800 Å) with a characteristic appearance as already mentioned have been first observed in cells of Rous sarcoma grown *in vitro* (Claude *et al.*, 1947). Since then a considerable amount of impressive work has been carried out in pioneering studies on metal-shadowed virus particles present in the blood of diseased chickens (Beard, 1963). Electron microscopy of ultrathin sections of particles inside the cells extracellularly located and in the blood of chickens (Bernhard *et al.*, 1958), as well as in purified preparations has revealed the internal structure of these particles (Bernhard, 1960). Similar structure with even greater detail has been shown in Rous sarcoma virus particles by Epstein (1957, 1958, 1960), and by Epstein and Holt (1958). The particles are composed of a central dense nucleoid, surrounded by a zone of low electron density which in turn is surrounded by a membrane; this membrane is surrounded by an electron-lucent zone and two outer membranes.

The results of studies combining treat-

Only biological tests of the extracts of the pancreas, showing the described morphological changes in apparently normal embryos and chickens, and in chickens infected with the myeloblastosis virus can determine the type of virus involved. It remains to be ascertained whether in the particular findings (Zeigel, 1961) or in those with the pancreas of infected chickens (Heine *et al.*, 1963), the virus of visceral lymphomatosis (RPL-12) or myeloblastosis was involved.

It is now known that erythroblastosis and visceral lymphomatosis develop in chickens maintained in contact with chickens bearing Rous sarcoma virus (Burmester *et al.*, 1960b). In addition, as already mentioned, the RPL-12 strain of lymphomatosis virus induces erythroblastosis (Burmester, 1952) and the BAI strain A of myeloblastosis virus induces visceral lymphomatosis, osteopetrosis, and nephroblastoma (Burmester *et al.*, 1959b). It would be of interest to ascertain whether the virus particles can be identified with the BAI-A strain myeloblastosis virus by the adenosine triphosphatase reaction. This, however, may be difficult as apparently only some cells show production of this enzyme, while other cells of chickens infected with the myeloblastosis strain A virus, such as chondrocytes and cells which lead to collagen formation present in nephroblastoma, fail to show the enzyme production (Heine *et al.*, 1962a).

The failure to observe virus particles in the pancreas of chickens with erythroblastosis induced by either strain R or ES4, need not necessarily indicate that the particles are not present in this organ. It may, of course, be an outcome of cell-type response to these two particular strains of avian tumor viruses.

Liver

Chickens with extensive hepatic lymphomatosis (massive parenchymal infiltration with lymphoid cells) induced by BAI strain A myeloblastosis virus show virus particle formation by budding of plasma membrane of lymphoid cells and in microvilli of liver parenchymal cells at the bile canalicular border (de Thé *et al.*, 1963b). In chickens with myeloblastosis, virus particles have been found in the lumen of canaliculi but no budding has been observed. Budding and virus particles appeared to be related to virus concentration in the invading lymphoid tissue or in the circulating blood plasma of myeloblasts. No budding or virus particles could be found in the liver with extensive lymphomatosis induced by transplanted nephroblastoma (de Thé *et al.*, 1963b).

The myeloblastosis virus induces, as already mentioned, a broad spectrum of neoplasias in chickens (Burmester *et al.*, 1959b) and non-neoplastic response in the thymus gland (de Thé *et al.*, 1963a; Arvy *et al.*, 1963) and in the pancreas (Heine *et al.*, 1963) in the infected chickens. It appears that the liver cells reveal a similar phenomenon of budding to that observed in the cells of the pancreas of infected and of some normal chickens, and also in the cells of normal chick embryos (Zeigel, 1961).

It is of interest that the hepatic cells showing the budding phenomenon show no evidence of hyperplasia or any cytological alterations (de Thé *et al.*, 1963b). Thus, it is possible in the virus-host cell reaction to have virus synthesis with or without neoplasia.

RETICULAR CELLS AND MACROPHAGES

The presence of avian tumor viruses in the cells of the thymus gland and of the pancreas as well as in the cells of other organs has now been ascertained. These cells do not participate directly in the neoplastic process, but nevertheless they are the seat of tumor virus synthesis. Similarly, reticular cells and macrophages of the spleen, bone marrow, and other tissues contain the virus and apparently are the seat of virus synthesis in myeloblastosis (Dmochowski *et al.*, 1958b; Parsons *et al.*, 1959), in erythroblastosis (Benedetti and Bernhard, 1958; Benedetti and Leplus, 1958; Dmochowski *et al.*, 1958a, 1959b; Heine *et al.*, 1961; Iwakata, 1958; Iwakata and Amano, 1958) and in visceral lympho-

matosis (Dmochowski *et al.*, 1959c). These cells appear to participate in virus synthesis more actively and more frequently than the respective tumorous cells of the erythroid, myeloid, or lymphoid series, but not exclusively, as reported by Beard (1963). Macrophagelike cells in Rous sarcoma cells grown *in vivo* or *in vitro* show changes (Beard, 1963) similar to those in macrophages studied in all forms of chicken leukosis. There is no need to accept the contention that macrophages or reticular cells have neoplastic traits, when concluding that these cells may also serve as seats of virus formation. It has indeed been proposed that macrophages may be the site of maturation of virus liberated in incomplete form by other cells in erythroblastosis (Iwakata and Amano, 1958).

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The results of studies combining treat-

ment with enzymes and electron microscopy have demonstrated that treatment with ribonuclease, under suitable conditions, leads to the removal of the core of virus particles present in Rous sarcoma tumors. A conclusion was therefore reached that this virus is an RNA-carrying virus (Epstein and Holt, 1958; Yamaguchi, 1962).

The virus particles, especially particles found outside the cells, vary in size and appearance, especially those found in nephroblastoma (Dmochowski, 1961, 1963; Dmochowski et al., 1960, 1961; Heine et al., 1962). This variation in size does not appear to be an artifact and may have some, as yet unknown, significance (Dmochowski et al., 1961). Nevertheless, virus particles present in the cytoplasmic inclusions or gray bodies (viroplasts) for the most part show a remarkable uniformity in size (800 Å) and spherical appearance, at least as far as can be judged by the techniques available at the present time. They are composed of a nucleoid, electron-lurent zone and outer double membrane. A quantitative correlation between the virus particles present in Rous sarcoma cells and tumor-inducing property has been obtained (Haguenau et al., 1958; Epstein, 1958, 1960). This is most encouraging as morphology alone can not serve as a criterion for the identification of a virus (Dmochowski, 1960b; Haguenau, 1960; Haguenau and Beard, 1962).

There is no doubt that too much emphasis cannot be placed on the sizes of virus particles as they appear in sections of tissues or high-speed centrifugal pellets of virus preparations. With this proviso, a comparison of the sizes and appearances of virus particles in tissues from different chicken neoplasms prepared in an identical manner can be made. Even in such uniformly prepared tissues or high-speed centrifugal pellets the particles of a certain strain may vary in size, shape, and internal structure. This is obviously not entirely the result of preparation procedures, but may indicate various stages in virus

particle formation and maturation. Calculations from sedimentation data of BAI strain A myeloblastosis virus (Sharp and Beard, 1954), indicate the size value of 1100 Å for the virus in hydrated state. It is of interest that at least some extracellular virus particles in the spleen of chickens with myeloblastosis (granuloblastosis) and in nephroblastoma are of the same size or are even larger. Therefore, the size and even the appearance of virus particles has to be taken with reservations. It is subject to revision as the various biophysical and electron microscope techniques of preparation of biological specimens are developed and improved.

The BAI strain A virus has been found to show strong activity to dephosphorylate adenosine and inosine triphosphates (Beard, 1963). The myeloblastosis virus particles in purified preparations also carry the enzyme (Green and Beard, 1955). This enzyme activity is proportional to the number of particles shown in the electron microscope study of the plasma of chickens with myeloblastosis or in tissue culture fluids from myeloblastosis grown in vitro (Beard, 1963). It is due to the inclusion of plasma membrane in the coat of the particles (Beard, 1963). Sedimentation, electrophoresis, and immunological studies (Beard, 1963) have demonstrated an intimate association of the enzyme activity with the virus. There is no evidence to suggest the possible biological significance of the association of the enzyme activity with the BAI strain A virus. Although probably not unique, this phenomenon nevertheless is of great interest, especially in view of the observation that loci of virus synthesis (viroplasts) in the neoplastic cells (myeloblasts) exhibit similar enzyme activity (Beard, 1963). This enzyme activity is also present in the budding plasma membrane of epithelial cells of nephroblastoma (de Thé et al., 1962) and of the thymus cells of chickens with myeloblastosis (Beard, 1963). Nevertheless, plasma membranes of the cartilage cells and chondrocytes do not show enzyme activity (de Thé et al., 1962)

and virus particles liberated by these cells do not show the enzyme (de Thé et al., 1962).

This enzyme activity is specific for the myeloblastosis virus (Beard, 1963). It is all the more of interest as the erythroblastosis virus and other chicken tumor viruses (Beard, 1963) have not shown a similar association with any enzyme activity. All previous studies which showed an association of enzyme activity with particles of other viruses were found to be erroneous, when improved purification procedures failed to confirm these studies.

In ultrathin sections of purified preparations of BAI strain A myeloblastosis virus, condensations of electron dense material have been observed with an appearance of either granules or filaments (Beard et al., 1963). A study of the appearance of myeloblastosis and of erythroblastosis virus (Beard et al., 1963; Bonar et al., 1963) following staining with potassium phosphotungstate (Brenner and Horne, 1959) has revealed an essential similarity in appearance betwen these two viruses and that of Rous sarcoma virus studied by the same technique (Dourmashkin et al., 1962; Dourmashkin and Simmons, 1961). The particles are spherical with knobs which surround the outer membrane. It is of interest that in some sections avian tumor viruses found in tissues of apparently normal chickens (Zeigel, 1961) and of infected chickens (Heine et al., 1963) appear surrounded by spines or protrusions which may correspond to those seen on particles stained negatively with potassium phosphotungstate.

Originally, virus particles of widely varying forms (spheroid, tadpole, spermlike) have been found present in shadow-cast preparations from the blood of chickens with BAI strain A virus (Sharp et al., 1952). They are similar to those of the Newcastle disease virus, observed under the same conditions (Cunha et al., 1947). Following the employment of a new technique based on drying of viruses on the surface of agar (Sharp et al., 1952) which led to the diffusion of water and salt, only spherical particles have been observed in shadow-cast preparations from the blood of chickens infected with BAI strain A virus. These studies have demonstrated that the tailed and pleomorphic forms of at least some viruses result from surface tension and salt concentration during drying of the virus particles (Beard et al., 1955). Similar conditions apparently result in a similar appearance of myeloblastosis BAI strain A virus (Beard et al., 1963), Rous sarcoma virus particles (Dourmashkin and Simmons, 1961), the mammary tumor virus (Dmochowski et al., 1963; Lyons and Moore, 1962), Gross mouse leukemia virus (Dmochowski et al., 1963) and presumably of other mouse leukemia viruses (Dalton et al., 1962; Zeigel and Rauscher, 1963). These tail-like and pleomorphic forms of the virus particles can largely be prevented by the resuspension in a proper diluent or by the employment of the sedimentation technique on agar (Sharp et al., 1952).

Treatment of BAI strain A virus particles with saponin leads to the disintegration of the outer membrane and to the appearance of structures 200–400 Å in size (Bonar et al., 1963) similar to those which have hemagglutinating properties in fowl plague (Waterson et al., 1961) and influenza virus (Horne and Waterson, 1960; Hoyle et al., 1961). Similar treatment of Rous sarcoma virus particles has revealed the structure of the outer membrane of the particles and of what may be the internal components of the virus (Dourmashkin and Simmons, 1961).

CONCLUSIONS

The electron microscope studies have demonstrated that the viruses of avian leukosis and other tumors cannot be distinguished morphologically from one another. Constantly accumulating evidence appears to indicate an essential similarity in the site of virus replication within the cells of all avian tumors and a considerable similarity in the relationship of these viruses to the constituents of cells of other

infected chicken organs, not directly involved in the neoplastic process, as well as to the constituents of cells of apparently normal chickens. The cells show viroplasm or viral matrix formation, cytoplasmic inclusions or viroplasts and the budding of plasma membrane. However, there appear to be quantitative differences in the type of response of cells of different origin to infection with the different virus strains. The apparent differences in the process of myeloblastosis virus synthesis in the thymus gland, kidney, and other cells may conceivably be only an expression of quantitative differences which may be revealed by further extensive electron microscope studies or they may be a real expression of the reaction of the different cell types to infection with the same virus. Nevertheless, some cells, such as macrophages, and reticular and epithelial cells apparently are capable of synthesizing avian tumor viruses in a manner similar to that of the cells (myeloblasts, erythroblasts, lymphoblasts) which respond by unrestricted proliferative growth. As some types of cells are capable of perpetuating the avian tumor viruses while other types of cells respond by neoplastic behavior, the level at which electron microscopy is now looking at virus infected cells is obviously not

capable of giving an indication of the morphological changes which lead to neoplasia. Electron microscopy with further improvements in specimen preparation and resolution may reveal even finer details of changes which occur in the cells infected with neoplastic viruses. It is apparent however that only if combined with other approaches electron microscopy may conceivably give some indication of the nature of the neoplastic process. While this may be a long-term goal, a more immediate aim appears to be the electron microscope study, preferably combined with cytochemical studies of suitably prepared specimens of one type of neoplasia induced by viruses of different strains, for example, a study of erythroblastosis induced by strains R, ES4, RPL-12, or by Rous sarcoma. Such studies may help to determine whether the morphological changes observed in erythroblastosis of the infected chickens will be similar or will reveal the quantitative differences characteristic of the particular virus strains. There is no doubt that correlative biological, cytochemical, and electron microscope studies will shed much light on virus-host cell relationship not only in avian neoplasia but in other types of cancer of viral origin.

REFERENCES

Atvy, L., Sommer, J. R., de Thé, G., Heine, U., Ishiguro, H., Beard, D., and Beard, J. W.: 1963. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. III. Histologic alteration and adenosine triphosphatase activity of the thymus of chickens with myeloblastosis. Jour. Nat. Cancer Inst. 30:401.

Baluda, M. and Jamieson, P. P.: 1961. *In vivo* infectivity studies with avian myeloblastosis virus. Virology 14:33.

Beard, J. W.: 1956. Virus of avian myeloblastic leukosis. Poultry Sci. 35:203.

——: 1963. Virus growth and their etiologic agents. *Advances in Cancer Research.* Academic Press, Inc., New York and London, p. 2.

——, Bonar, R. A., Heine, U., de Thé, G., and Beard, D.: 1961. Studies on the biological, biochemical and biophysical properties of avian tumor viruses. 17th Annual Symposium on Fundamental Cancer Research. Viruses, Nucleic Acids, and Cancer. The Williams and Wilkins Co., Baltimore. P. 344.

——, Sharp, D. G. and Eckert, E. A.: 1955. Tumor viruses. Advances in Virus Research 3:149.

Beaudreau, G. S., Becker, C., Bonar, R. A., Wallbank, A. M., Beard, D., and Beard, J. W.: 1960. Virus of avian myeloblastosis. XIV. Neoplastic response of normal chicken bone marrow treated with the virus in tissue culture. Jour. Nat. Cancer Inst. 24.395.

——, Becker, S., Sharp, D. G., Painter, J. G., and Beard, J. W.: 1958. Virus of avian myeloblastosis. XI. Release of the virus by myeloblasts in tissue culture. Jour. Nat. Cancer Inst. 20 351

Benedetti, E. L.: 1957. Présence de corpuscules indentiques à ceux du virus de l'erythroblastose aviaire chez l'embryon du poulet et les poussins normaux. Bul. du Cancer 44:473.

——, and Bernhard, W.: 1958. Recherches ultrastructurales sur le virus de la leucémie érythroblastique du poulet. Jour. Ultrastructure Research 1:309.

———, and Leplus, R.: 1958. Cytologie de l'erythroblastose aviaire (étude au microscope électronique). Rev. d'Hematol. 13:199.

———, Bernhard, W., and Oberling, C.: 1956. Présence de corpuscules d'aspect virusal dans des cellules spléniques et médullaires de poussins leucémiques et normaux. Compt. rend. Acad. Sci. 242:2891.

Bernhard, W.: 1958. Electron microscopy of tumor cells and viruses. A review. Cancer Res. 18:491.

———: 1960. The detection and study of tumor viruses with the electron microscope. Present facts and problems as seen by a morphologist. Cancer Res. 20:712.

———, Bonar, R. A., Beard, D., and Beard, J W : 1958. Ultrastructure of virus of myeloblastosis and erythroblastosis isolated from plasma of leukemic chickens Proc. Soc. Exper. Biol. and Med. 97:48.

———, Haguenau, F., and Leplus, R.: 1955 Coupes ultrafines d'éléments sanguines et de ganglions lymphatiques études au microscope electronique Rev. d'Hematol 10:267.

———, Oberling, C., and Vigier, P.: 1956. L'ultrastructure du particules de virus du sarcome de Rous et leur rapport avec le cytoplasme de cellules tumorales. Bull. du Cancer 43:407.

Bonar, R. A., Beaudreau, G. S., Sharp, D G., Beard, D., and Beard, J W: 1957 Virus of avian erythroblastosis. V. Adenosine triphosphatase activity of blood plasma from chickens with the disease. Jour. Nat. Cancer Inst. 19 909

———, Heine, U., Beard, D., and Beard, J W.: 1963. Virus of avian myeloblastosis. XXIII. Morphology of virus and comparison with strain R (erythroblastosis). Jour. Nat. Cancer Inst. 30.949.

———, Parsons, D. F., Beaudreau, G. S., Becker, C., and Beard, J. W: 1959. Ultrastructure of avian myeloblasts in tissue culture. Jour. Nat. Cancer Inst. 23:199.

———, Weinstein, D., Sommer, J. R., Beard, D, and Beard, J W.: 1960. Virus of avian myeloblastosis. XVII. Morphology of progressive virus myeloblast interactions in vitro. Nat. Cancer Inst. Monograph No. 4. P. 251.

Brenner, S., and Horne, R. W.: 1959. A negative staining method for high resolution electron microscopy of viruses. Biochem. et Biophys. Acta 34:103.

Bryan, W. R.: 1959. Quantitative biological experimentation in the virus and cancer fields. Jour. Nat. Cancer Inst. 22:129.

Burmester, B. R.: 1947. Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell-free preparations. Cancer Res. 7:786.

———: 1952. Studies on fowl lymphomatosis. Ann. N.Y. Acad. Sci. 54:992.

———: 1957. Transmission of tumor inducing avian viruses under natural conditions. Texas Symposia on Cancer Res. 15.92.

———, Fontes, A. K., and Walter, W. G.: 1960a. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. III. Influence of host age and route of inoculation. Jour. Nat. Cancer Inst. 24:1423

———, Fontes, A. K., and Walter, W. G.: 1960b. Contact transmission of Rous sarcoma. Jour. Nat. Cancer Inst. 25:307.

———, Fontes, A. K., Waters, N. F., Bryan, W. R., and Groupe, V.: 1960c. The response of several inbred lines of White Leghorns to inoculation with viruses of strain RPL-12 visceral lymphomatosis-erythroblastosis and of Rous sarcoma. Poultry Sci. 39:199.

———, Gross, M. H., Walter, W. G., and Fontes, A. K.: 1959a. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. II. Host-virus interrelation affecting response. Jour. Nat. Cancer Inst. 22:103.

———, Sharpless, G. R., and Fontes, A. K.: 1960d. Virus isolated from avian lymphomas unrelated to lymphomatosis virus. Jour. Nat. Cancer Inst. 24:1443.

———, Walter, W. G., Gross, M. A., and Fontes, A. K.: 1959b. The oncogenic spectrum of two "pure" strains of avian leukosis. Jour. Nat. Cancer Inst. 23:277.

Claude, A., Porter, K. R., and Pickels, E. G.: 1947. Electron microscope study of chicken tumor cells. Cancer Res. 7:421.

Crawford, L. V.: 1960. A study of the Rous sarcoma virus by density gradient centrifugation. Virology 12:143.

———, and Crawford, E. M.: 1961. The properties of Rous sarcoma virus purified by density gradient centrifugation. Virology 13.227.

Cunha, R., Weil, M. L., Beard, D, Taylor, A. R., Sharp, D. G., and Beard, J. W: 1947. Purification and characteristics of the Newcastle disease virus (California strain) Jour. Immunol. 55 69.

Dalton, A. J., Haguenau, F., and Moloney, J. B.: 1962. Morphology of particles associated with murine leukemia as revealed by negative staining: preliminary report. Jour. Nat. Cancer Inst. 29:1177.

de Thé, G., Heine, U., Sommer, J. R., Arvy, L., Beard, D., and Beard, J. W.: 1963a. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. IV. Ultrastructural characters of the thymus in myeloblastosis and of the adenosine triphosphatase activity of thymic cells and associated virus. Jour. Nat. Cancer Inst. 30:415.

de Thé, G., Ishiguro, H., Beard, D., and Beard, J. W.: 1963b. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. VII. Elaboration of virus by non-neoplastic hepatic cells. Jour. Nat. Cancer Inst. 31:717.

——, Ishiguro, H., Heine, U., Beard, D., and Beard, J. W.: 1963c. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. VI. Ultrastructural aspects of adenosine triphosphatase activity of nephroblastoma cells and virus. Jour. Nat. Cancer Inst. 30:1257.

——, Nowiкоff, A. B., Heine, U., and Beard, J. W.: 1962. Preliminary studies of cytochemistry of avian tumors. Proceedings of the fifth International Congress for Electron Microscopy. New York, Academic Press, Inc. 2:2.

Dmochowski, L.: 1960a. Viruses and tumors in the light of electron microscope studies: a review. Cancer Res. 20.977.

——: 1960b. Viruses and tumors. Science 133.551.

——: 1960c. The viral etiology of leukemia. Progress in Medical Virology. Karger, Basel/New York 3:363.

——: 1961. Sites and modes of virus replication. Symposium on Nuclear-Cytoplasmic Relationships. Colorado State University, Fort Collins, Colorado. P. 93.

——: 1963. The electron microscope view of virus-host relationship in neoplasia. Progress in Experimental Tumor Research, Karger, Basel/New York. 3:36.

——, and Grey, C. E.: 1957. Electron microscopy of tumors of known and suspected viral etiology. Texas Symposia on Cancer Research 11:256.

——, and Grey, C. E.: 1958. Studies on submicroscopic structure of leukemias of known and suspected viral origin: a review. Blood 13:1047.

——, Grey, C. E., and Burmester, B. R.: 1959a. Studies on the submicroscopic structure of chicken leukosis: lymphomatosis, erythroblastosis, and granuloblastosis. Acta Internat. Union contra cancrum 15.780.

——, Grey, C. E., and Burmester, B. R.: 1959b. Submicroscopic morphology of avian neoplasms IV. Studies on erythroblastosis of strain RPL-12. Proc. Soc. Exper. Biol. and Med. 100.517.

——, Grey, C. E., Burmester, B. R., and Fontes, A. K.: 1958a. Submicroscopic morphology of avian neoplasms. I. Studies on erythroblastosis. Proc. Soc. Exper. Biol. and Med. 98:662.

——, Grey, C. E., Burmester, B. R., and Gross, M. A.: 1959c. Submicroscopic morphology of avian neoplasms. III. Studies on visceral lymphomatosis. Proc. Soc. Exper. Biol. and Med 100.514

——, Grey, C. E., Burmester, B. R., and Walter, W. G.: 1958b. Submicroscopic morphology of avian neoplasms. II. Studies on granuloblastosis (myeloblastosis). Proc. Soc. Exper. Biol. and Med. 98 666

——, Grey, C. E., Burmester, B. R., and Walter, W. G.: 1960 Electron microscopic studies of chicken renal adenocarcinoma. Jour. Applied Physics 31:1859.

——, Grey, C. E., Burmester, B. R., and Walter, W. G.: 1961. Submicroscopic morphology of avian neoplasms. V. Studies on nephroblastoma. Texas Reports on Biology and Medicine 19 545.

——, Grey, C. E., Padgett, F., Langford, P. L., and Burmester, B. R.: 1964. Submicroscopic morphology of avian neoplasms VI. Comparative studies on Rous sarcoma, visceral lymphomatosis, erythroblastosis, myeloblastosis, and nephroblastoma. Texas, Reports on Biology and Medicine 22:20.

——, Grey, C. E., Padgett, F., and Sykes, J. A.: 1963. Studies on the structure of the mammary tumor-inducing virus (Bittner) and of leukemia virus (Gross). Seventeenth Annual Symposium on Fundamental Cancer Research: Viruses, Nucleic Acids, and Cancer. The Williams and Wilkins Co., Baltimore, P. 85.

Dourmashkin, R. R., Daugherty, R. M., and Harris, R. J. C.: 1962. Electron microscopic observations on Rous sarcoma virus and cell membranes. Nature (London) 194:1116.

——, and Simmons, P. J.: 1961. The ultrastructure of Rous sarcoma virus. Jour. Ultrastructure. Res. 5:505.

Engelbreth-Holm, J., and Rothe-Meyer, A.: 1932. H. Über den Zusammenhang zwischen den Verschiedenen Huhnerleukoseformen (Anämie-Erythroblastose-Myelose). Acta Path. et Microbiol. Scand. 9.312.

——, and Rothe-Meyer, A.: 1935. On the connection between erythroblastosis (haemocytoblastosis), myelosis, and sarcoma of chickens. Acta Path. et Microbiol. Scand. 12:352.

Epstein, M. A.: 1957. The fine structural organization of the Rous tumor cells. Jour. Biophys. and Biochem. Cytol. 3.851.

——: 1958. Observations on the Rous sarcoma virus: purification and identification of the particles from solid tumors. Brit. Jour. Cancer 12:248.

——: 1960. Constitution of tumor virus. Nat. Cancer Inst. Monograph No. 4. P. 53.

——, and Holt, S. J.: 1958. Observations on the Rous virus: integrated electron microscopical and cytochemical studies of fluorocarbon purified preparations. British Jour. Cancer 12.363.

Febvre, H. L., and Benedetti, E. L.: 1958. Mise en évidence grâce à la culture de tissu de particules de virus latents dans l'embryon de poulet. Buf du Cancer 45:454.

Gaylord, W. H.: 1955. Virus-like particles associated with the Rous sarcoma as seen in sections of the tumor. Cancer Res. 15:80.

Green, I., and Beard, J. W.: 1955. Virus of avian erythromyeloblastic leukosis. VI. Properties of the enzyme associated with the virus in dephosphorylating adenosine triphosphate. Jour. Nat. Cancer Inst. 15:1217.

Gross, A. M., Burmester, D. R., and Walter, W. G.: 1959. Pathogenicity of a viral strain (RPL-12) causing avian visceral lymphomatosis and related neoplasms. I. Nature of the lesions. Jour. Nat. Cancer Inst. 22:83.

Haddad, M. N., Weinstein, D., Bonar, R. A., Beaudreau, C. S., Becker, C., Beard, D., and Beard, J. W.: 1960. Virus of avian myeloblastosis. XV. Structural loci of virus synthesis and adenosine triphosphatase activity by electron and light microscopy of myeloblasts from tissue culture. Jour. Nat. Cancer Inst. 24:971.

Haguenau, F.: 1960. Significance of ultrastructure in virus-induced tumors. Symposia on Tumor Viruses. Nat. Cancer Inst. Monograph No. 4. P. 211.

——, and Beard, J. W.: 1962. The avian sarcoma leukosis complex; its biology and ultrastructure. Tumors Induced by Viruses. Dalton, A. J., and Haguenau, F. eds. Academic Press, New York and London, 1:1–60.

——, Dalton, A. J., and Moloney, J. B.: 1958. A preliminary report of electron microscopic and bioassay studies on the Rous sarcoma I virus. Jour. Nat. Cancer Inst. 20:633.

——, Febvre, H. L., and Arnoult, J.: 1960. Ultrastructure du virus du sarcome de Rous cultivé in vitro. Compt. rend. Acad. Sci. 250:1747.

——, Febvre, H. L., and Arnoult, J.: 1960b. Ultrastructural morphology of Rous sarcoma grown in vitro. Perspectives in Virology, Pollard, M., ed. Burgess, Minneapolis, Minn. 2:160.

——, Febvre, H. and Arnoult, J.: 1962. Mode de formation intracellulaire du virus du sarcome de Rous. Étude ultrastructurale. Jour. Microscopie 1:445.

Hall, W. J., Beam, C. W., and Pollard, M.: 1941. Transmission of fowl leukosis through chick embryos and young chicks. Am. Jour. Vet. Res. 2:272.

Heine, U., Beaudreau, G. S., Becker, C., Beard, D., and Beard, J. W.: 1961. Virus of avian erythroblastosis. VII. Ultrastructure of erythroblasts from the chicken and from tissue culture. Jour. Nat. Cancer Inst. 26:359

——, de Thé, C., Ishiguro, H., Sommer, J. R., Beard, D., and Beard, J. W.: 1962a. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. II. Nephroblastoma (Wilms' tumor): Ultrastructure. Jour. Nat. Cancer Inst. 29:41.

——, de Thé, C., Ishiguro, H., and Beard, J. W.: 1962b. Morphologic aspects of Rous sarcoma elaboration. Jour. Nat. Cancer Inst. 29:211.

——, de Thé, G., Beard, D., and Beard, J. W.: 1963. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. V. Elaboration of virus by pancreas of chickens inoculated with the agent. Jour. Nat. Cancer Inst. 30:817.

Horne, R. W., and Waterson, A. P.: 1960. A helical structure in mumps, Newcastle disease, and Sendai viruses. Jour. Molecular Biol. 2:75.

Hoyle, L., Horne, R. W., and Waterson, A. P.: 1961. The structure and composition of the myxoviruses. II. Components released from the influenza virus particle by ether. Virology 13.448.

Ishiguro, H., Beard, D., Sommer, J. R., Heine, U., de Thé, G., and Beard, J. W.: 1962. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. I. Nephroblastoma (Wilms' tumor): Gross and microscopic pathology. Jour. Nat. Cancer Inst. 29:1.

Iwakata, S.: 1958. Electron microscopic observations of chicken erythroblastosis. II. Report with special reference to varied features of macrophages as reservoirs of the virus. Ann. Rept. Inst. of Virus Res. Kyoto Univ.) 1:221.

——, and Amano, S.: 1958. Causative virus of chicken erythroblastosis and the modes of intercellular proliferation of this virus section under the electron microscope and the modes of intercellular proliferation of this virus in various forms. Acta Haematol. Japan 21 154.

Johnson, E. D.: 1911. Fowl leukosis, manifestations, transmission, and etiological relationship of various forms. Virginia Agr. Exper. Sta. Tech. Bul. 76.3.

Lyons, M. J., and Moore, D. H.: 1962. Purification of the mouse mammary tumor virus. Nature (London) 191.1141.

Malmgren, R. A., Fink, M. A., and Mills, W.: 1960. Demonstration of the intracellular localization of Rous sarcoma virus antigen by fluorescent labeled antiserum. Jour. Nat. Cancer Inst. 24.995.

Mannweiler, K., and Bernhard, W.: 1958. L'ultrastructure du myxosarcome de Fujinami. Bul. Cancer 45 223

Mellors, R. C.: 1960. Tumor cell localization of the antigens of the Shope papilloma virus and the Rous sarcoma virus. Cancer Res. 20 744.

Noyes, W. F.: 1960. Development of Rous sarcoma virus antigens in cultured chick embryo cells. Virology 12 468.

Olson, C., Jr.: 1961. A transmissible lymphoid tumor of the chicken. Cancer Res. 1:584.

Parsons, D. F., Beaudreau, G. S., Becker, C., Bonar, R. A., and Beard, J. W.: 1959. Cell multiplication, virus liberation, and ultrastructure of avian myeloblasts in tissue culture. Acta Union Intern contra Cancer 15:826.

——, Painter, J. C., Beaudreau, G. S., Becker, C., and Beard, J. W.: 1958. Tissue culture and circulating myeloblasts of avian leukemia studied in electron micrographs of ultrathin sections. Proc. Soc. Exper. Biol. and Med. 97:839.

Knöller, C., Haguenau, F., Golde, A., and LaCour, F.: 1956. L'ultrastructure de l'endothéliome de Murray-Begg. Le problème de l'identification de son agent causal. Bul. Cancer 43:10.

Rubin, H.: 1960. A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus. Proc. Nat. Acad. Sci. U.S. 46:1105.

——: 1961. The nature of virus induced cellular resistance to Rous sarcoma virus. Virology 13:200.

Sharp, D. G., and Beard, J. W.: 1954. Virus of avian erythromyeloblastic leukosis. IV. Sedimentation, density, and hydration. Biochem. et Biophys. Acta 14:12.

——, Eckert, E. A., Beard, D., and Beard, J. W.: 1952. Morphology of the virus of avian erythromyeloblastic leucosis and a comparison with the agent of Newcastle disease. Jour. Bacteriol. 63:151.

Sommer, J. R., Weinstein, D., Becker, C., Beaudreau, G. S., Beard, D., and Beard, J. W.: 1962. Virus of avian myeloblastosis. XIX. Protein, polysaccharide, lipid, and nucleic acid of myeloblasts and cytidine uptake in vitro. Jour. Nat. Cancer Inst. 28:75.

Thorell, B.: 1958 Induktion von Nierentumoren durch Leukaemievirus. Schwedische Pathologenvereinigung, Sondersjukhuset, Stockholm, December 7, 1958. Zentr. allgem. Pathol. u. Path. Anat. 98 514.

——: 1960 Modifications of the host reaction towards leukemia virus. An experiment with virus induced leukemia and kidney tumor in the chicken. Proc. 7th Intern. Congress of the Internat Soc. of Hematology 3:582

Waters, N. F.: 1951. Mortality from lymphomatosis and other causes among inbred lines of White Leghorns Poultry Sci 30.531.

——, Burmester, B. R., and Walter, W. G.: 1958 Genetics of experimentally induced erythroblastosis in chickens. Jour. Nat. Cancer Inst. 20:1245.

Waterson, A. P., Rott, R., and Schäfer, W.: 1961. The structure of fowl plague virus and virus N. Zeitschrift f. Naturschig. 16b:154.

Weinstein, D., Sommer, J. R., Beaudreau, G. S., Becker, C., Bonar, R. A., and Beard, J. W.: 1960. Virus of avian myeloblastosis XVIII. Fixation of myeloblasts and phosphatase activity of loci of virus synthesis (viroplast). Jour. Nat. Cancer Inst. 25:1421.

Yamaguchi, J.: 1962. Localization of RNA, protein, and lipid in Rous sarcoma virus by electron microcytochemistry. Proc. Fifth Internat. Congress for Electron Microscopy, Breese, S. S., Jr., ed. Academic Press, New York 2:1.

Zeigel, R. F.: 1961. Morphological evidence for the association of virus particles with the pancreatic acinar cells of the chick. Jour. Nat. Cancer Inst. 26:1011.

——, and Rauscher, F. J.: 1963. Electron microscopic and bioassay studies on a murine leukemia virus (Rauscher). preliminary report Jour. Nat. Cancer Inst. 30:207.

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20

Infectious Bronchitis

Avian infectious bronchitis is an acute, highly contagious, respiratory disease of chickens. The disease may occur in all age groups, but in the United States and Canada it is particularly a problem in adult laying flocks. Infectious bronchitis was first reported by Shalk and Hawn (1931), who had observed the disease in North Dakota in the spring of 1930. It soon became widespread, as indicated by the reports of others: namely, Beaudette and Hudson (1933), Bushnell and Brandly (1933), Beach (1934), and Beach and Schalm (1936). Infectious bronchitis has also been reported to occur in Israel (Komorov et al, 1941), the Netherlands (Swierstra, 1947; Richter, 1955; Bijlenga, 1956), England (Asplin, 1948), Germany (Fritzsche, 1952), Japan (Sato et al., 1955a; 1955b; Kawakubo et al., 1958), Italy (Galassi, 1956; Papparella et al., 1956; Petek, 1956; Petek and Corazzola, 1957), Greece (Ayfantis, 1956), Brazil (Hipólito, 1957),

France (Brion et al., 1959), and Hawaii (Raggi, 1960).

Etiology. The causative agent of infectious bronchitis is a filterable virus. The name *Tarpeia pulli* has been proposed for the virus (Merchant and Packer, 1956). Reagan et al. (1948) have estimated the virus to be about 65–135 mμ (millimicrons) in size. Filtration studies through gradocol membrane filters also placed the particle size within this range, 70–105 mμ (Hofstad, 1957). The virus passes the Seitz EK pad, all grades of Berkefeld filters, and the Selas 06 filter. It can be grown in the developing chicken embryo, where the virus causes stunting or death of the embryo after a few serial passages.

Infectious bronchitis virus has been grown in cell cultures of chicken embryo kidney cells and fibroblasts (Chomiak et al., 1958; Gunningham, 1960; Pette, 1960; and Kawamura et al., 1961). Only the embryo-adapted strains, such as the Beaudette

strain, will grow in cell cultures. Growth is detected by a cytopathic effect which can be neutralized by specific immune serum. Mallman and Cunningham (1963) found, in allantoic fluid infected with infectious bronchitis virus, a factor which enhanced the attachment and rapid formation of monolayers of chicken embryo cell cultures on glass surfaces. Carbo and Cunningham (1959) found infectious bronchitis virus to agglutinate chicken red blood cells after having been treated with 1 per cent trypsin for 3 hours at 37° C.; however, the reaction could be inhibited by normal as well as immune serum. Muldoon (1960) reported that the hemagglutinin could be released after treating the virus with 1 per cent trypsin for 30 minutes at 56° C. The treated virus could be stored for 3 weeks at −65° C. without loss of activity.

Infected tissues stored in 50 per cent glycerin retain their activity for at least 80 days in a refrigerator (Beach, 1918). In this medium, tissues can be shipped to a laboratory for diagnosis without refrigeration. Bronchitis virus in phosphate buffer at pH 7.79 remained active for 170 days at 4° C. and for 142 days at pH 8.2 in undiluted allantoic fluid (Cunningham and Stuart, 1947a). These authors (1946) found a laboratory strain of infectious bronchitis to be destroyed by common disinfecting agents such as 1 per cent phenol, 1 per cent liquor cresolis saponatus, 1:10,000 potassium permanganate solution, 70 per cent ethyl alcohol, and 1 per cent formalin within the three minute contact period. Quiroz and Hanson (1958) reported bronchitis virus to resist 1 per cent HCl or pH 2 for 1 hour at room temperature, a treatment which inactivated laryngotracheitis and fowl pox viruses. They also found 1 per cent phenol to have no effect on bronchitis virus exposed for 1 hour at room temperature, a treatment which inactivated the B_1 strain of Newcastle disease virus. Ether (20 per cent) reduced the titer but did not inactivate the virus, as was also found by Petek and Corazzola (1958). Most strains of bronchi-

tis virus, diluted 1:100 in 20 per cent horse serum broth, were destroyed after 15 minutes' exposure to 56° C.; however, there was considerable variation among the strains studied (Hofstad, 1956b). Singh (1960) studied the thermostability of bronchitis virus at 56° C. He found a bimodal inactivation at 56° C. indicating existence of two phases, an O (original) phase which was thermostable and a D (derivative) phase which was thermolabile. Virus strains in high embryo passage, such as the Beaudette strain, are in the D phase. The O phase was also found to be more resistant to formalin than the D phase. Cunningham and Stuart (1947b) found that freezing and thawing had no harmful effect on the virus. The virus has remained active for at least 19 years in the lyophilized state at 3° C. It has remained active for at least 7 years stored at −25° C. to −30° C. in the form of infective egg fluid (Hofstad, 1957). Buddha (1956) found 10 per cent glucose to give a stabilizing effect on bronchitis virus in the frozen and lyophilized state.

Transmission and incubation period. The incubation period of infectious bronchitis is 18 to 36 hours, depending on the dosage and route of inoculation. Chickens exposed to an aerosol of undiluted infective egg fluid regularly have tracheal rales within 18 to 24 hours. Natural spread requires about 36 hours or more. The disease spreads rapidly among birds in a flock. Susceptible chickens can be readily infected by intranasal or intratracheal inoculation of tracheal exudate or lung tissue suspension from an infected chicken. Air-borne transmission has been demonstrated experimentally (Levine and Hofstad, 1947), and field observations indicate that transmission through the air takes place readily. Hofstad and Kenzy (1950) found natural air-borne transmission a reliable means of exposing birds in challenge experiments.

The epizootiology of infectious bronchitis is not well understood. The recurrence of the disease year after year on the same farm may indicate that some re-

covered birds remain carriers of the virus, thus perpetuating the infection. However, attempts to demonstrate carriers under experimental conditions have not been successful. Following inoculation of a group of chickens with bronchitis virus, Fabricant and Levine (1951) were unable to detect the presence of virus in tracheal swabs and in the yolk of eggs collected from the recovered birds after 36 days. In nine trials where bronchitis-recovered birds were placed in contact with susceptible chickens, Hofstad (1947) was unable to demonstrate the presence of virus longer than 35 days after recovery. Pette (1959) has recovered bronchitis virus from the cloacal contents up to 24 days after experimental oral infection.

Symptoms. The most characteristic symptoms in young chicks are nasal discharge, gasping, rales, and coughing. The chicks tend to crowd under the hover to keep warm. Wet eyes are commonly seen, and swollen sinuses may be observed occasionally. As the disease progresses, many chicks become weak and depressed. Infectious bronchitis in chicks under 2 weeks of age may cause permanent damage to the oviduct, resulting later in false layers (Broadfoot et al., 1956). However, this is not the only cause of this condition according to Hutt et al. (1956). Mortality in very young chicks may be as high as 25 per cent, but in chickens over 6 weeks of age mortality is negligible. Prince et al. (1962) found feed consumption and weight gain significantly reduced by bronchitis infection.

In chickens over 6 weeks of age and in adult birds the outstanding symptoms are gasping, tracheal rales, and coughing. A nasal discharge is usually not observed. Blood counts taken during the course of the disease reveal a leukopenia during the
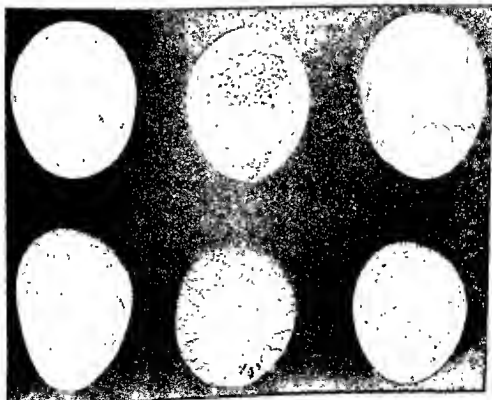
FIG. 20.1 — Thin-shelled, rough, and misshapen eggs laid by hens during an outbreak of infectious bronchitis. (Van Roekel, Univ. of Mass.)

first 2 days followed by a leukocytosis which declines after the seventh day to reach normal by the fifteenth day (Machado, 1951).

In laying flocks, production will decline, and misshapen, rough, and soft-shelled eggs (Fig. 20.1) may be found (Gordeuk and Bressler, 1950; Van Roekel et al., 1951; Hill and Lorenz, 1956; Sevoian and Levine, 1957). Broadfoot and Smith (1954) found egg production reduced 25 per cent, the number of unsettable hatching eggs increased 92 per cent, and hatchability reduced 7 per cent in outbreaks of bronchitis studied. Laying of poor quality eggs may continue in some flocks even after recovery of full production. Birds affected in the latter part of their laying year usu-

ally have a marked drop in egg production and some degree of molt. Such flocks require long periods of time to recover production. Pullets in good condition which have started to lay may suffer only a slight drop and regain normal production within a few weeks after recovery from respiratory symptoms. The course of the disease is usually one to two weeks, although it is not uncommon for a few birds in the flock to have symptoms for longer periods.

Pathology. On necropsy of affected chicks, a serous or catarrhal exudate is observed in the trachea. There is a catarrhal or a fibrinous inflammation of the air sacs. In chicks that die, yellowish, caseous plugs may be found in the lower trachea and bronchi. Small areas of pneumonia
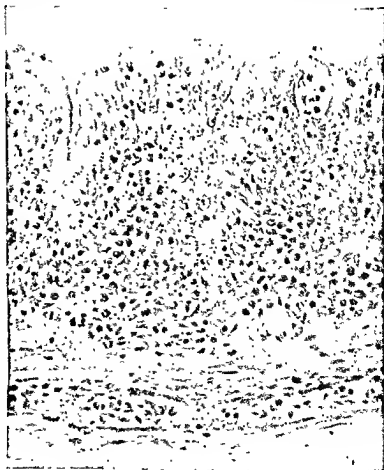
FIG. 20.2 — Portion of trachea from an experimentally infected bird 72 hours following inoculation, showing thickened tracheal mucous membrane due to cellular infiltration and edema. ×450.

around the large bronchi may be present occasionally. Very young chicks also have a catarrhal inflammation of the nasal passages and sinuses, causing nasal discharge, wet eyes, and occasionally swollen sinuses. As chicks grow older, this lesion is less common, and in birds over two months of age there is seldom gross involvement of the upper nasal passages and sinuses.

Hofstad (1945) found a thickening of the tracheal mucosa and submucosa due to edema and diffuse cellular infiltration as the principal microscopic lesions in the respiratory tract (Figs. 20.2 and 20.3). There was no interruption of the continuity of the tracheal epithelium, and the lumen contained an exudate in which cellular elements were usually sparse or absent (Fig. 20.4). No inclusion bodies have been observed. On necropsy of infected chickens that are in production, fluid yolk material is found in the abdominal cavity. This is a nonspecific

change and is found in other diseases causing a marked drop in production. Sevoian and Levine (1957) studied the gross and microscopic pathology of the reproductive tract of infected chickens and chickens subjected to physiological stress (removal of feed and water). Oviduct length and weight were markedly reduced in both groups, but return to normal required 21 days for the infected group but only 11 days for the group under stress. Ovarian regression was similarly affected. Microscopically the height of the cellular epithelium lining the oviduct was sharply reduced and the cells became cuboidal in shape with some loss of cilia. Dilation of the glands occurred in half the oviducts studied. In the lamina propria and intertubular stroma of the oviducts, lymphocytic foci and cellular infiltration were present. Significantly fewer changes of a similar nature were seen in the group under stress.

Immunity. Chickens that have recovered

FIG. 20.3 — Portion of trachea from a field case of infectious bronchitis, showing cellular infiltration and edema of the mucosa and submucosa, vascular congestion, vacuolation of the epithelium, and hemorrhage in the submucosa.

from infectious bronchitis are resistant to intratracheal inoculations as soon as symptoms have subsided. It requires about 3 weeks for most birds to have a high level of antibodies following exposure to bronchitis virus (Fabricant, 1951). After recovery, antibodies can be demonstrated for one year or longer. Most flocks that have experienced an outbreak of bronchitis are immune for at least 12 months. Observations by Van Roekel *et al.* (1950), however, have suggested that immunity to the disease may decline sufficiently for reinfection to occur in some flocks following exposure to the virus. Jungherr and Terrell (1948) have demonstrated that eggs laid by recovered hens will carry antibodies which will later be absorbed by the hatched chick. Antibodies were found in the serum of the chick for 3 weeks. Such passive antibodies, however, did not serve to completely protect the chicks against

natural exposure to infectious bronchitis (Hofstad and Kenzy, 1950).

Diagnosis. The diagnosis of infectious bronchitis must be based upon isolation of the virus or by demonstrating an ascending antibody titer against a known strain of bronchitis virus.

It is difficult to attempt even a presumptive diagnosis in the early stage of the disease because its clinical manifestations are similar to those of Newcastle disease and laryngotracheitis. After the disease has progressed sufficiently, knowledge of the symptoms, mortality, and duration makes possible a presumptive diagnosis. Fabricant (1950) has found that a typical clinical history of infectious bronchitis coupled with a negative hemagglutination inhibition test for Newcastle disease justifies a presumptive diagnosis of infectious bronchitis. This would be valid in areas
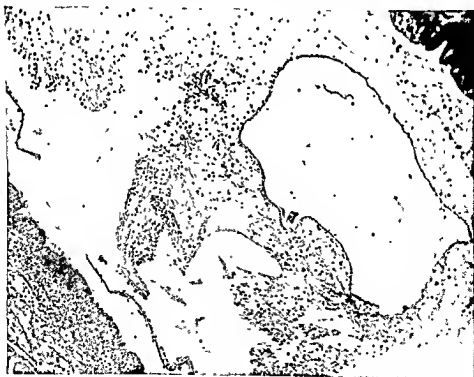
FIG. 20.4 — Portion of trachea from a field case of infectious bronchitis, showing exudate containing scattered cellular elements in the lumen of the lower trachea. ×100.

where these two respiratory diseases were the only ones commonly found.

Virus isolation. Infectious bronchitis virus may be isolated by intratracheal inoculation of susceptible chicks with a broth suspension of lung and trachea collected from an infected bird during the early stages of the disease. Symptoms of tracheal rales will follow an incubation period of 18 to 36 hours. This short incubation period is characteristic of infectious bronchitis. Inoculation of both susceptible and immune chickens would give definite information for a diagnosis. However, this procedure is not commonly carried out.

Isolation of the virus is most frequently done in the embryonating chicken egg. This is achieved by inoculating a suspension of lung and trachea into the allantoic cavity of 9- to 11-day-old embryonating eggs. The virus may also be found in other tissues to a lesser extent, such as the aqueous humor (Flowers et al., 1957) and spleen, kidney, bursa, air sac, and pancreas (Hofstad, 1962). Contaminating bacteria in the inoculum are controlled by either filtering through a bacteriological filter or by adding a mixture of antibiotics. The presence of infectious bronchitis virus in the embryonating egg following inoculation is somewhat difficult to discern in the initial passage since there is little change in the majority of the embryos inoculated. Dwarfing of a few embryos by some strains and survival of most of the embryos in the initial passage is characteristic of bronchitis virus. Its presence can be definitely detected, however, by the intratracheal inoculation of susceptible chicks with allantoic fluid collected after a postinoculation period of 48 to 96 hours. If bronchitis virus is present, the chicks will have tracheal rales after a period of 18 to 36 hours.

The information obtained following the above procedures, together with a typical flock history and symptoms, will permit a diagnosis of infectious bronchitis within 3 or 4 days. Results of further egg passages of the isolated virus and the detection of antibodies in the serum collected from the inoculated chicks three weeks after recovery would positively confirm the diagnosis.

The effect of infectious bronchitis virus on the embryo has been studied in detail by Beaudette and Hudson (1937), Delaplane and Stuart (1939, 1941), Fabricant (1949), Loomis et al. (1950), and Raggi et al. (1960). While some strains may produce dwarfing of a few embryos during the initial egg passage, stunting, curling, and death of the embryo can be seen more frequently in the second, third, and succeeding passages. The typical dwarfed embryo (Fig. 20.5) will be seen more consistently as the number of passages increases. Simpson (1958) found the incubation temperature to markedly alter the virus growth, virulence, and population stability in embryonated eggs. One strain of virus reached the same maximum titer in eggs incubated at 34° C. or 38° C., but it required at least 30 hours longer to attain this level at 34° C. The virus was lethal for embryos at 38° C., but only 20 per cent were killed at 34° C.

The characteristic embryo changes are seen several days after inoculation of the virus. During candling, only slight movement of a dwarfed embryo may be observed. In opening the air-cell end of the egg, the embryo is seen curled into a spherical form with the thickened amnion closely adherent to the embryo. The yolk sac appears shrunken, and an increased volume of usually clear allantoic fluid is present. A consistent internal lesion of the bronchitis-infected embryo is the persistence of the mesonephros containing urates. This lesion appears to be associated with the stunting of the embryo and is not specific for bronchitis infection. Another lesion found in embryonating eggs inoculated with nonlethal isolates of bronchitis virus is the thickened amnion and adjacent layer of the allantois covering the stunted embryo. The beginning of this lesion can usually be detected on the third day after inoculation. It likewise is not a pathognomonic lesion since it can also be observed following inoculation of

eggs with lentogenic strains of Newcastle disease virus.

The microscopic lesions in the embryo have been studied by Loomis *et al.* (1950) They found perivascular cuffing in the livers of about one-third of the infected embryos. Extensive necrosis and congestion of the kidneys were also prominent. Edema was present in the amnion and chorioallantoic membrane.

The embryo mortality following inoculation of bronchitis virus increases with the number of serial passages. Beaudette and Hudson (1937) observed very few dead embryos during the first six passages when inoculations were made on the chorio-allantoic membrane. However, in the seventh and later passages the majority of embryos died. Delaplane and Stuart (1941), likewise making the inoculation on the chorio-allantoic membrane, observed a greater mortality with each succeeding egg transfer. In early passages

the mortality occurred late, but in subsequent transfers the embryos died by the end of the second day. Hofstad (1952) observed an average mortality, following inoculation into the allantoic sac, of less than 10 per cent during the first passage, 61 per cent by the fifth serial passage, and 83 per cent by the ninth serial passage in a study of field strains of bronchitis virus.

Infectious bronchitis virus propagated in the embryonating egg gradually loses its ability to infect chickens. This loss of pathogenicity for chickens occurred after 89 egg transfers with a Rhode Island strain mentioned by Delaplane and Stuart (1941). Hofstad (1952) confirmed these findings with another strain propagated via the allantoic sac route. Hoekstra and Rispens (1960b) found their strain of bronchitis virus to possess some pathogenicity after 120 embryo passages. Larose and Van Roekel (1961) observed strain 42 (Beaudette) to be pathogenic for chickens
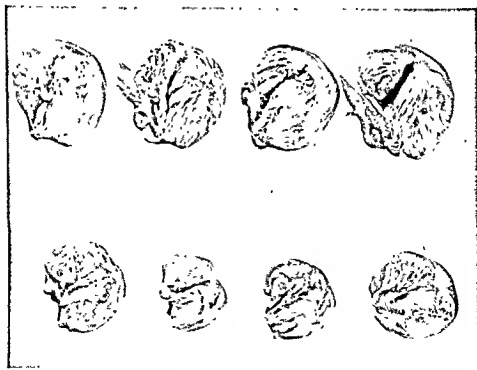
FIG. 20.5 — Comparison of normal 16-day-old embryos (above) and dwarfed, infected embryos of same age (below). (Hofstad and Bauriedel, Iowa State University.)

after 300 serial transfers in embryos. The loss of pathogenicity for the chicken is accompanied by a loss of immunogenic properties.

The distribution of the virus of infectious bronchitis in the embryonating egg has been studied by Cunningham and El Dardiry (1948). Following inoculation into the allantoic sac, the highest concentration of virus is recovered from the chorio-allantoic membrane followed in order by the allantoic fluid, amnionic fluid, and the liver. The highest concentration of 10^7 embryo lethal doses was detected 36 hours after inoculation. A decrease of titer resulted if eggs were left in the incubator after death of the embryo. Groupé (1949) detected an interfering substance in the allantoic fluid of such eggs. The substance was not present when infected eggs were removed immediately or not more than two hours after death of the embryo. Hitchner and White (1955) studied the growth curve of a vaccine strain (20–30 embryo passages) of infectious bronchitis virus in eggs and found maximum virus concentration 24 to 30 hours after allantoic route inoculation. The embryo lethal (Beaudette) strain of virus was found to reach its maximum titer in 12 hours.

Detection of antibodies against bronchitis virus. Infected chickens are bled in the initial stages of the disease and again in 3 weeks. Both samples of sera are tested for antibodies against bronchitis virus. Demonstration of a low antibody titer in the serum collected during the initial stage, followed by a high titer after recovery, constitutes a diagnosis of infectious bronchitis.

The usual method of detecting antibodies is to set up ten-fold dilutions in broth of a known embryo-adapted bronchitis virus, such as the Beaudette strain. The virus titer is determined by inoculating each ten-fold dilution into a group of four or five 9- to 12-day-old embryonating eggs. Each egg receives 0.05 ml. When the virus dilutions are being made, a portion of each dilution of virus is

mixed in a separate tube with an equal amount of suspect serum. The two portions are mixed by shaking the tubes. Each mixture of virus dilution plus serum is then inoculated into a set of four or five embryonating eggs, using 0.1 ml. Most of the neutralization occurs immediately with maximum neutralization in 15 minutes (Cunningham, 1957). Neutralization of the virus is exponential (Page and Cunningham, 1962).

Following inoculation, the eggs are incubated and candled daily for one week to remove eggs containing dead embryos. The 50 per cent end point of embryo mortality in each series can then be determined by the method of Reed and Muench (1938). The difference between these end points represents the neutralizing capacity of the serum. For example, if the mortality end point of the virus titration was in the 10^{-6} dilution and the end point of the serum-virus mixture was in the 10^{-3} dilution, then the neutralizing capacity of the serum would be 1,000 neutralizing doses in 0.05 ml. of serum. The neutralizing capacity of normal chicken serum may range from 0 to a maximum of 36 neutralizing doses (Cunningham, 1951), while serum of a bronchitis-recovered bird should have 100 or more neutralizing doses (Fabricant, 1951). Woernle (1959) and Woernle and Brunner (1960) have demonstrated bronchitis antibodies by the agar gel precipitation technique. This test is done by placing antigen (chorio-allantoic membrane suspension from infected embryos) in one well in an agar, and suspected antiserum in an adjacent well and observing the precipitin lines between the two wells. Woernle (1960) found no difference between German strains, the Beaudette strain, and the Connecticut and Massachusetts strains by the agar gel precipitin test.

Brumfield and Pomeroy (1957) have applied the direct complement fixation test to infectious bronchitis diagnosis. Jeon (1962) has standardized the details of the test and studied the principal mechanism involved in the technique. Brown and Schminle

(1962) have described an indirect hemagglutination test for infectious bronchitis. The virus is adsorbed onto tannic acid-treated, horse red blood cells and after washing are mixed with the suspected serum. If the serum has antibodies it reacts with the antigen and causes clumping of the red cells. Vazington (1962) also described a similar indirect hemagglutination test; however he used sheep erythrocytes instead of horse red cells.

Treatment. There is no specific treatment for infectious bronchitis. In flocks of young chicks it is helpful to increase the temperature of the room as well as of the brooder. Overcrowding should be corrected. In laying flocks drafts should be eliminated, and warm, moist mashes should be given to encourage the birds to eat. Anything the poultryman can do to keep up feed consumption in the flock should be encouraged to avoid excessive loss in weight in the birds. Dusting or spraying so called cold remedies are not worth their cost. Recovery takes place as the birds acquire an immunity to the virus. If the disease is complicated with chronic respiratory disease or air-sac infection, treatment with the broad-spectrum antibiotics may be indicated. (See Chapter 13.)

Prevention and control. The best preventive is strict isolation of the flock, along with sound management practices, such as adding only day-old chicks as replacement stock and rearing them in isolation. The poultry house should be properly ventilated. Even on farms with sound management practices, infectious bronchitis may occur, particularly in heavily populated areas. This has brought on the necessity of using immunization procedures in the control of the disease.

The first immunization procedure used was started about 1941 in the New England states (Van Roekel et al., 1950). It consisted of inoculating a small portion of the birds in a flock with a field strain of virus and allowing natural spread to the rest of the flock. This was usually done at 7 to 15 weeks of age when the disease would produce the least economic loss to the poultryman. After recovery from the infection, the flock would be immune to bronchitis through the laying year.

This type of vaccination procedure using pathogenic field strains has been replaced with modified live virus vaccines (Brandt et al., 1952; Luginbuhl and Jungherr, 1952; Crawley, 1953, 1955; Hofstad, 1954, 1956; Hoekstra, 1960; and Hoekstra and Rispens, 1960a). These modified vaccines use strains of virus that have undergone 25 or more embryo passages to reduce their pathogenicity and spreading ability. It should be emphasized that while these modified strains at present are safer to use, they are still capable of spreading, and in the very young chick without passive antibodies and in laying flocks in high production they may produce undesirable results.

The replacement flock should be vaccinated during the growing period preferably at 3 to 4 months. In broilers it is necessary to immunize at an early age. If the chicks possess passive antibodies derived from the yolk, they can be inoculated at a few days of age. These antibodies lessen the severity of the infection but Raggi and Lee (1958) found that the young age and passive antibodies were important factors in preventing a satisfactory immune response to live virus vaccine. Chicks which do not possess passive antibodies will tolerate the vaccine best after three weeks of age.

Several methods of administration of bronchitis vaccines have come into use—the spray or aerosol (Crawley, 1953; Hofstad, 1954), the dust (Markham et al., 1955; Price et al., 1955), and drinking water (Luginbuhl et al., 1955) procedures. These mass vaccination techniques have become very popular with the poultryman because they are labor saving. The bronchitis vaccines have also been combined with Newcastle disease vaccines as an added convenience and apparently without interference in the immune response from each vaccine (Markham et al., 1956). However, interference has been demonstrated between these two viruses in certain combinations (Luginbuhl and Jung-

herr, 1953; Hanson *et al.*, 1956), and it would seem advisable that whenever possible and practical the bronchitis immunization should be administered separately.

The immunity which can be expected from commercial bronchitis vaccines is variable (Raggi and Bankowski, 1956). There is definite loss of immunogenicity with increased attenuation of bronchitis virus (Crawley, 1955; Hofstad 1956c). Raggi and Lee (1957) found a direct relationship between SN titers and response to challenge in only one of three vaccines studied. There was a noticeable difference with the other two vaccines. This tended to confirm the reports of Jungherr *et al.* (1956), Hofstad (1956a, 1958) who found strain differences using reciprocal serum neutralization tests. Hofstad (1961), however, later found that cross-challenge results did not agree with serum neutralization results. There was some cross-immunity between isolates which had previously been found antigenically different by reciprocal serum neutralization tests. The field prevalence of variant bronchitis strains is not known; however, the Massachusetts type or the typical field strain apparently will induce the best immunity to challenge with heterologous strains (Raggi, 1960; Hofstad, 1961). In general, infectious bronchitis is being satisfactorily controlled.

Complications resulting from the use of infectious bronchitis vaccines in flocks free of *Mycoplasma gallisepticum* are rare. However, when this organism is present either in active or latent form, immunization with bronchitis virus may kindle the infection, resulting in so-called air-sac disease. The end result is that the bronchitis vaccine "take" is extended in an exaggerated form beyond the 10- to 14-day period seen in uncomplicated cases. In the case of broilers this means cull birds, mortality, a delay in marketing the flock, and a grading down of the carcasses. In replacement flocks it may mean a delay in coming into production and an increased number of culls. The effects of both bronchitis virus and *Mycoplasma gallisepticum* in chickens have been studied by Adler *et al.* (1962) and Blake (1962).

Attempts to produce an inactivated virus vaccine have resulted in loss of immunogenic properties following inactivation by formalin (Delaplane and Stuart, 1939) and ultraviolet light (Hofstad, 1952). Christian and Mack (1957) used beta-propiolactone to inactivate bronchitis virus. A concentration of 0.25 per cent at 37° C. for 60 minutes was minimum treatment for inactivation. Some protection to challenge and a slight rise in serum antibodies resulted in birds receiving 1 and 2 ml. doses. Woernle (1961) reported some success with an adsorbed bronchitis vaccine inactivated by 0.2 per cent formalin. Evaluation of the vaccine was based on the agar diffusion precipitin test. Embryo-adapted strains of bronchitis virus which have lost their infectivity for chickens have been of no practical immunizing value. Beaudette *et al.* (1956) were unable to demonstrate immunity in the 18 days following intramuscular inoculation of the 24th embryo passage of a live bronchitis strain of virus.

REFERENCES

Adler, H. E., McMartin, D. A., and Ortmayer, H.: 1962. The effect of IBV on chickens infected with *M. gallisepticum*. Avian Dis. 6(3):267.

Asplin, F. D.: 1948. Identification of infectious bronchitis of chickens in England. Vet. Record 60 (Pt. 2):485.

Ayfantis, S.: 1956. La bronchite infectieuse des poussens en Grece. Hellen Kleniatraikes Hezeiess Delt. Ser. B 21:3.

Beach, J. R.: 1934. Coryza and other respiratory infections in chickens. Proc. 12th Intern. Vet. Cong. 3:144.

———: 1948. Infectious bronchitis. *In Diseases of Poultry*, Second edition, H. E. Biester and L. H. Schwarte. Iowa State College Press, Ames, Iowa. P. 455.

———, and Schalm, O. W.: 1936. A filterable virus distinct from that of laryngotracheitis, a cause of a respiratory disease of chicks. Poultry Sci. 15:199.

Beaudette, F. R., Bivins, J. A., Burd, H. E., and Hudson, C. B.: 1956. Effect of passive immunity, mode of inoculation, and dose of virus on immunity response in bronchitis. Vet. Med. 51:519.

———, and Hudson, C. B.: 1953. Newly recognized poultry disease. No. Am. Vet. (March) 14:50.

———, and Hudson, C. B.: 1937. Cultivation of the virus of infectious bronchitis. Jour. Am. Vet. Med. Assn. 90:51.

Bijlenga, G.: 1956. Infectious bronchitis in chicks in the Netherlands. (Trans. title.) Tijdschr. Diergeneesk. 81 43.

Blake, J. T.: 1962. Effects of experimental chronic respiratory disease and infectious bronchitis on pullets. Am. Jour. Vet. Res. 23(95):847.

Brandt, C. D., Van Roekel, H., and Peck, H. A.: 1952. An egg-propagated immunizing agent for the control of infectious bronchitis of chickens. Poultry Sci. 31(6):1004.

Brion, A., Fontaine, M., and Fontaine, M. P.: 1959. La bronchite infectieuse des gallines. Rec. Méd. Vet. 135:435.

Broadfoot, D. I., Pomeroy, B. S., and Smith, W. M., Jr.: 1956. Effects of infectious bronchitis in baby chicks. Poultry Sci. 35:757.

———, and Smith, W. M., Jr.: 1954. Effects of infectious bronchitis in laying hens on egg production, percent unsettable eggs and hatchability. Poultry Sci. 33(5):653.

Brown, W. E., Schmittle, S. C., and Foster, J. W.: 1962. A tannic acid modified hemagglutination test for infectious bronchitis of chickens. Avian Dis. 6.99.

Brumfield, H. P., and Pomeroy, B. S.: 1957. Direct complement fixation by turkey and chicken serum in viral systems. Proc. Soc. Exper. Biol. & Med. 94:146.

Bushnell, L. D., and Brandly, C. A.: 1933. Laryngotracheitis in chicks. Poultry Sci. 12:55.

Buthala, D. A.: 1956. Some properties of the avian bronchitis virus. Ph.D. thesis, Iowa State College.

Corbo, L. J., and Cunningham, C. H.: 1959 Hemagglutination by trypsin-modified infectious bronchitis virus. Am. Jour. Vet. Res. 20.876

Chomiak, T. W., Luginbuhl, R. E., and Jungherr, E. L.: 1958. The propagation and cytopathogenic effect of an egg-adapted strain of infectious bronchitis virus in tissue culture. Avian Dis. 2.456.

Christian, R. T., and Mack, W. N.: 1957 An experimental infectious bronchitis virus vaccine inactivated with betapropiolactone. Poultry Sci. 36:1177.

Crawley, J. F: 1955. The extent and control of infectious bronchitis in Canada. Proc. Am. Vet. Med. Assn. 1955 342.

———. 1955. Present status of infectious bronchitis immunization. Proc. Am. Vet. Med. Assn. 1955.345.

Cunningham, C. H.: 1951. Newcastle disease and infectious bronchitis neutralizing antibody indexes of normal chicken serum. Am. Jour. Vet. Res. 12:129.

———. 1957. Symposium on immunization against infectious bronchitis virus. Am. Jour. Vet. Res. 18 648

———. 1960. Recent studies on the virus of infectious bronchitis. Am. Jour. Vet. Res. 21:498.

———, and El Dardiry, A. H.: 1948. Distribution of the virus of infectious bronchitis of chickens in embryonated chicken eggs. Cornell Vet. 38.381.

———, and Stuart, H. O.: 1946. The effect of certain chemical agents on the virus of infectious bronchitis of chickens. Am. Jour. Vet. Res. 7:466.

———, and Stuart, H. O.: 1947a. The pH stability of the virus of infectious bronchitis of chickens. Cornell Vet. 37:99.

———, and Stuart, H. O.: 1947b. Cultivation of the virus of infectious bronchitis of chickens in embryonated chicken eggs. Am. Jour. Vet. Res. 8:209.

Delaplane, J. P., and Stuart, H. O.: 1939. Studies of infectious bronchitis. R.I. Agr. Exper. Sta., Bull. 273.

———, and Stuart, H. O.: 1941. The modification of infectious bronchitis virus of chickens as a result of propagation in embryonated chicken eggs. R.I. Agr. Exper. Sta., Bul. 284.

Fabricant, J.: 1949. Studies on the diagnosis of Newcastle disease and infectious bronchitis of fowls. II. The diagnosis of infectious bronchitis by virus isolation in chick embryos. Cornell Vet. 39 414.

———. 1950. III. The differential diagnosis of Newcastle disease and infectious bronchitis. Cornell Vet. 40:39.

———. 1951. IV. The use of the serum neutralization test in the diagnosis of infectious bronchitis. Cornell Vet. 41:68.

———, and Levine, P. P.: 1951. The persistence of infectious bronchitis virus in eggs and tracheal exudates of infected chickens. Cornell Vet. 41:240.

Flowers, A. I., Grumbles, L. C., and Delaplane, J. P.: 1957. Isolation of infectious bronchitis virus from the aqueous humor of chickens. Southwestern Vet. 10(2).135.

Fritzsche, K.: 1932. Die infektiöse bronchitis des Huhnes. Berliner Münchener tierärztl. Wochenschr. 65:299.

Galassi, D.: 1956. Bronchite infettiva del pollo: isolamento del virus e tentativo di vaccinazione con un ceppo a virulenza attenuata. Atti Soc. Ital. Delle Sci. Vet. 10:717.

Gordeuk, S., Jr., and Bressler, G. O.: 1950. Infectious bronchitis, its effect on rate of egg production and egg quality. Progress report No. 36. Pa. State Coll. of Agr., State College, Pa.

Groupé, V.: 1949. Demonstration of an interference phenomenon associated with infectious bronchitis virus (IBV) of chickens. Jour. Bact. 58.23.

Hanson, L. E., and Alberts, J. O.: 1959. Factors affecting interference with Newcastle disease. Am. Jour. Vet. Res. 20:352.

———, White, F. H., and Alberts, J. O.: 1956. Interference between Newcastle disease and infectious bronchitis viruses. Am. Jour. Vet. Res. 17-294.

Hill, R. W., and Lorenz, F. W.: 1956. Studies on egg changes following avian respiratory diseases. I. Diseases associated with egg changes. Poultry Sci. 35:409.

Hipólito, O.: 1957. Avian infectious bronchitis in Brazil. Arch. Esc. Vet. Minas Gerais 10:131.

Hitchner, S. B., and White, P. G.: 1955. Growth-curve studies of chick-embryo-propagated infectious bronchitis virus. Poultry Sci. 34:590.

Hoekstra, J.: 1960. Infectieuze bronchitis bij pluimvee II. Praktijkervaringen met de sterkwerkende entstof. Tijdschr. Diergeneesk. 85:320.

———, and Rispens, B.: 1960a. Infectieuze bronchitis bij pluimvee. I. Laboratoriumexperimenten met een sterkwerkende entstam. Tijdschr. Diergeneesk. 85:279.

———, and Rispens, B.: 1960b. Infectieuze bronchitis bij pluimvee. III. De ontwikkeling van een mild werkendvaccin. Tijdschr. Diergeneesk 85:598.

Hofstad, M. S.: 1945. A study of infectious bronchitis in chickens. I. The pathology of infectious bronchitis. Cornell Vet. 35.22.

———: 1947. A study of infectious bronchitis in chickens. IV. Further observations on the carrier status of chickens recovered from infectious bronchitis. Cornell Vet. 37:29.

———: 1952. Infectious bronchitis — Progress Report of Veterinary Medical Research Institute, Ames, Iowa, 1952:48.

———: 1954. Infectious bronchitis — Progress Report of Veterinary Medical Research Institute, Ames, Iowa. 1954:10.

———: 1956a. Infectious bronchitis — Progress Report of Veterinary Medical Research Institute, Ames, Iowa. 1956:13.

———: 1956b. Stability of avian infectious bronchitis virus at 56° C. Cornell Vet. 46:122.

———: 1956c. Immunization of chickens against infectious bronchitis using an embryo-passaged attenuated strain of virus. Vet. Med. 51:464.

———: 1957. Unpublished data.

———: 1958. Antigenic differences among isolates of avian infectious bronchitis virus. Am. Jour. Vet. Res. 19:740.

———: 1961. Antigenic and immunological studies on several isolates of avian infectious bronchitis virus. Avian Dis. 5:102.

———: 1962. Unpublished data.

———, and Kenzy, S. G.: 1950. Susceptibility of chicks hatched from recovered hens to infectious bronchitis. Cornell Vet. 40.87.

Hutt, F. B., Goodwin, K., and Urban, W. D.: 1956. Investigations of nonlaying hens. Cornell Vet. 46:257.

Jeon, Y. S.: 1962. Modified complement fixation test of avian infectious bronchitis virus. University Microfilm Inc., Ann Arbor, Mich. Dissertation abst. 23:400.

Jungherr, E. L., Chomiak, T. W., and Luginbuhl, R. E.: 1956. Immunologic differences in strains of infectious bronchitis. Proc. 60th. Ann. Meet. U.S. Livestock Sanit. Assn. 1956:203,

———, and Terrell, N. L.: 1948. Naturally acquired passive immunity to infectious bronchitis in chicks. Am. Jour. Vet. Res. 9:201.

Kawakubo, A., Kuba, N., and Nakamura, J.: 1958. Studies on infectious bronchitis of chickens. I. Isolation of virus and characters in isolated virus N.I.B.S. Bul. biol. Res. Tokyo 3:31.

Kawamura, H., Isogai, S., and Tsubahara, H.: 1961. Propagation of avian infectious bronchitis virus in chicken kidney tissue culture. Nat. Inst. Anim. Health Quart. Tokyo 1:190.

Komorov, A., and Grasovsky, V.: 1961. Notes on specific infectious respiratory disease affecting baby chicks (infectious bronchitis). Refu. Vet. Jour. 97:107.

Larose, R. N., and Van Roekel, H.: 1961. The effect of rapid embryo passage upon the infectious bronchitis virus. Avian Dis. 5:157.

Levine, P. P., and Hofstad, M. S.: 1947. Attempts to control air-borne infectious bronchitis and Newcastle disease of fowls with sternlamps. Cornell Vet. 37:204.

Loomis, L. N., Cunningham, C. H., Gray, M. L., and Thorp, F., Jr.: 1950. Pathology of the chicken embryo infected with infectious bronchitis virus. Am. Jour. Vet. Res. 11:215.

Luginbuhl, R. E., and Jungherr, E. L.: 1952. Brooder chick vaccination for infectious bronchitis. Poultry Sci. 31:924.

———, and Jungherr, E. L.: 1953. Simultaneous titration of Newcastle disease and bronchitis viruses in embryonating eggs. Poultry Sci. 32.941.

———, Jungherr, E. L., and Chomiak, T. W.: 1955. Administration of Newcastle disease virus and infectious bronchitis viruses through the drinking water. Poultry Sci. 34.1399.

———, and infectious bronchitis viruses through the drinking water and NDV on the blood cells of the chicken.

Machado, A. V.: 1951. The effect of infectious bronchitis on the graduate faculty of Cornell University. Thesis submitted to the graduate faculty of Cornell University.

Singh, I. P.: 1960. Some properties of IBV as determined by thermal and formalin inactivation. Ph.D. thesis. University Microfilm Inc., Ann Arbor, Mich. Dissertation abst. 22:2153.

Swierstra, D.: 1947. Bronchitis infectiosa bij kippen in Nederland. Tijdschr. Diergeneesk. 72:745.

Van Roekel, H., Bullis, K. L., Clarke, M. K., Olesuik, O. M., and Sperling, F. G.: 1950. Infectious bronchitis. Mass. Agr. Exper. Sta., Bul. 460.

———, Clarke, M. K., Bullis, K. L., Olesiuk, O. M., and Sperling, F. G.: 1951. Infectious bronchitis. Am. Jour. Vet. Res. 12:140.

Vasington, P. J.: 1962. Studies on passive hemagglutination with infectious bronchitis virus. University Microfilm Inc., Ann Arbor, Mich. Dissertation abst. 22 2949.

Woernle, H.: 1959. Diagnose der Infektiösen Bronchitis der Hühner mit Hilfe der Präzipitationsreaktion im festen Agarmedium. Monatsh Tierheilk. 11-154

———: 1960. Ein Beitrag zur Infectiön Bronchitis der Hühner. Monatsh. Tierheilk. 12:111.

———: 1961. Impfversuche mit Adsorbat-Vakzine bei der Infektiosen Bronchitis des Huhnes. Monatsh. Tierheilk. 13:156.

———, and Brunner, A.: 1960. Zur Epizootiologie der Infektiösen Bronchitis der Hühner. Tierarztl. Umschau 15:217.

QUAIL BRONCHITIS

Quail bronchitis, an acute respiratory infection of quail (*Colinus virginianus*, Linné), was reported by Olson (1950). The disease occurred on a state game farm in West Virginia during the 1949 hatching season. In 1956 a similar disease was noted in Texas by DuBose et al. (1958).

The symptoms of the disease were tracheal rales, sneezing and coughing, but no nasal discharge was observed. Nervous symptoms were occasionally observed. Mortality was from 50 to 80 per cent in the field outbreaks, however neither Olson (1950) nor DuBose and Grumbles (1959) was able to demonstrate much mortality in the experimental cases. The incubation period was reported to be from 4 to 7 days and the course of the disease, from 1 to 3 weeks.

Olson (1950) found that Chukar Partridges were not susceptible, however he was able to reisolate virus from chickens and turkeys after experimental inoculation. DuBose et al. (1958) found pheasants and "Japanese Quail" to be resistant to infection.

The etiology of quail bronchitis is a filterable virus which can be grown in thicken embryos, where it causes dwarfing of the embryo and a thickening of the amnion similar to that produced by infectious bronchitis virus of chickens. The tious bronchitis virus can be differentiated from infectious bronchitis virus by its ability to withstand 56° C. for 90 minutes whereas most strains of infectious bronchitis virus are inactivated after 15 minutes at 56° C. and all of them after 30 minutes (DuBose et al., 1960). It has also been demonstrated to be serologically distinct from infectious bronchitis virus.

Quail bronchitis virus can be stored for as long as 4 years at −20° C. (DuBose et al., 1958). The virus passes through the Seitz EK filter pad. Virus has been isolated from the brain from a few cases having nervous symptoms. DuBose and Grumbles (1959) also isolated virus consistently from the aqueous humor and they found it to be as good a source of virus as tracheal exudate.

Yates and Fry (1957) studied the relationship between quail bronchitis virus and an agent which they had isolated and described as a chicken embryo lethal orphan (CELO) virus. They found that CELO antiserum neutralized 10⁷ e.l.d. of quail bronchitis virus, thus establishing the similarity between these two agents. DuBose and Grumbles (1959) confirmed the similarity between the two agents and were able to reproduce the disease in quail by inoculation of either agent, and also demonstrated contact transmission with both agents. Yates and Fry (1957) have demonstrated neutralizing antibodies from the major poultry producing areas in the United States, and thus these viruses apparently are widespread among avian species.

DuBose, R. T., and Grumbles, L. C: 1959. The relationship between quail bronchitis virus and chicken embryo lethal orphan virus. Avian Dis. 3:321.

———, Grumbles, L. C, and Flowers, A. I: 1958. The isolation of a non-bacterial agent from quail with a respiratory disease. Poultry Sci. 37.654.

———, Grumbles, I. C., and Flowers, A. I.. 1960. Differentiation of quail bronchitis virus and infectious bronchitis virus by heat stability. Am. Jour. Vet. Res. 21:740.

Olson, N. O: 1950. A respiratory disease (bronchitis) of quail caused by a virus. Proc. U.S. Livestock Sann. Assn 171.

Yates, V. J., and Fry, D. E., 1957. Observations on a chicken embryo lethal orphan (CELO) virus. Am. Jour. Vet. Res. 19.657.

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21

Infectious Laryngotracheitis

The first outbreak of infectious laryngotracheitis, an acute, contagious, respiratory disease of chickens, was reported by May and Tittsler (1925). They first observed the disease in a flock of chickens in Rhode Island in October of 1923. Beach (1925) reported outbreaks of the disease occurring in California in 1924. In a survey by Hinshaw (1931a), reports of observations indicated that the disease may have existed some years prior to 1924; however, the disease became of economic importance in the United States in 1924. Some experiments by Gwatkin (1925) indicated that infectious laryngotracheitis was present in Canada. Seddon and Hart (1935, 1936) identified the disease in Australia in 1935. The first outbreak in Great Britain was recorded by Dobson (1935). Van Heelsbergen (1929) stated that the disease had been observed in Holland. Later the disease was reported from Sweden (Magnusson, 1940) and from Poland (Marek, 1948).

Some of the early investigators called the disease infectious bronchitis; however, the more appropriate name infectious laryngotracheitis was later recommended by the Special Committee on Poultry Diseases of the American Veterinary Medical Association, and this nomenclature was adopted (1931).

Etiology. The causative agent of infectious laryngotracheitis is a filterable virus (Beaudette, 1930; Beach, 1930, 1931a, 1931b; Gibbs, 1931a; Graham *et al.*, 1931). The virus has been named *Tarpeia avium* (Holmes, 1948). It is retained by the Berkefeld W filter but passes through the N filter with irregularity and through the Berkefeld V filter readily. Variable results have been reported using the Seitz filter pad. Filtering a 1:50 broth dilution of infected chorio-allantoic membrane suspension through a series of Selas filters, it was found that it passed through the 03 filter but irregularly passed the 04 filter. Bakos *et al.* (1962) found the virus to pass

through all grades of the Berkefeld candles and through gradacol membranes of 450 μ pore size. Watrach et al. (1959) found the virus to have an average diameter of 180 μ when purified preparations were studied by electron microscopy. In tissue sections they found the virus to be morphologically similar to the herpes group. Laryngotracheitis virus can be propagated in the embryonating chicken egg, where it causes primarily proliferative and necrotic lesions on the chorio-allantoic membrane (Burnet, 1934; Brandly, 1935, 1936).

The virus has been grown in cultures of respiratory epithelial cells, in fibroblast cells, and in kidney cells. (Atherton and Anderson, 1957; Chang et al., 1960; Chomiak et al., 1960; and Pulsford, 1960). The virus produces a cytopathic effect in which the cells undergo a granular degeneration into sharply limited masses. The eclipse phase is relatively long, particularly with the low virulent strains. Atherton and Anderson (1957) were unable to demonstrate virus in the fluid of growing cultures; however, Pulsford (1960) did demonstrate release of virus into the fluid of cell cultures with the strains of virus he used. Inclusion bodies were observed in cultures of respiratory epithelium but not in fibroblast cell cultures.

The resistance of the virus to certain physical and chemical factors has been studied by Schalm and Beach (1935). Virus in tracheal exudate exposed to 55° C. survived from 10 to 15 minutes. When infective tracheal exudate was suspended in 50 per cent glycerine in buffer at pH 7.4 and held in the dark, the infectivity was retained from 7 to 14 days at 37° C., from 35 to 42 days at 16° to 24.5° C., and as long as 217 days at 4° to 10° C. Virus in the trachea of dead fowls survived from 22 to 44 hours at 37° C., and from 30 to 60 days at 4° to 10° C. The infectivity of the virus is well preserved when dried from the frozen state (Goldhaft, 1961; Hofstad and Yoder, 1963). Beaudette et al. (1948) reported active virus in dried preparations stored almost 10 years in the refrigerator. The virus is destroyed by one-

half-minute exposure to 3 per cent cresol disinfectant and to 1 per cent solution of lye.

There is evidence of differences among strains of laryngotracheitis virus. Burnet (1936) found an enzootic Australian strain (Victorian) to be less readily neutralized by immune serum and to produce smaller lesions on the chorio-allantoic membrane than the more virulent, epizootic United States and New South Wales isolates. However, there appeared to be no qualitative antigenic difference between them. Pulsford (1953, 1954) confirmed the observations of Burnet when he found a strain of laryngotracheitis virus which was resistant to neutralization by antiserum, although it was capable of producing the disease with resulting antibodies against a classical strain. Cover and Benton (1957) and Satriano et al. (1957) have isolated strains of laryngotracheitis virus of low pathogenicity for chickens.

Susceptible hosts. The virus of laryngotracheitis has a distinct host specificity. Chickens are the usual host, but pheasants are also susceptible to the virus by inoculation or natural exposure (Kernohan, 1931a; Hudson and Beaudette, 1932a). The following species of birds have been found refractory to the virus: turkeys, starlings, quail, sparrows, ducks, pigeons, guinea fowl, doves, and crows. White rats, guinea pigs, mice, and rabbits are likewise resistant (Beach, 1931b; Brandly and Bushnell, 1934; Seddon, 1936). The embryonating eggs of the turkey and chicken are susceptible to the virus, while those of the duck, guinea fowl, and pigeon are refractory (Brandly, 1936).

Transmission and incubation period. Following natural exposure, the incubation period of laryngotracheitis varies from 6 to 12 days (Kernohan, 1930; Seddon and Hart, 1935). After intratracheal inoculation of infective material, symptoms occur in 2 to 4 days.

The natural route of infection is by way of the respiratory tract. Gibbs (1931b) was unable to produce the disease by feeding capsules of dried material, but when

the dried infective powder was placed in the litter, the chicks soon developed the disease. Laryngotracheitis can be produced by intravenous, subcutaneous, and intraperitoneal inoculation, although not consistently (Gibbs, 1933a). Bakos et al. (1962) were unable to produce symptoms following intracutaneous and intramuscular inoculation.

It is well established that carriers of the virus exist for long periods after a natural outbreak of the disease (Gibbs, 1931a, 1931b, 1932; Komarov and Beaudette, 1932). Only a few birds in each flock remain carriers, but these serve to perpetuate the disease whenever they come in contact with susceptible chickens. The method of detecting carriers is to swab the tracheas of recovered birds and to inoculate susceptible chickens. By this method, carriers have been found to eliminate virus for as long as 16 months after an outbreak.

Transmission of the disease indirectly by way of contaminated poultry crates and other equipment and by flying birds is possible, although it is probably of minor importance in the spread of laryngotracheitis. Kingsbury and Jungherr (1957) reported evidence of indirect transmission in fifteen outbreaks by means of rat, man, crow, and dog. Brandly and Bushnell (1934) were unable to demonstrate virus on the surface of eggs which were laid during an outbreak of the disease.

Symptoms. The acute, epizootic form of the disease usually spreads rapidly in a flock, and all or most of the birds become affected. Hinshaw (1931b) has recorded histories of some outbreaks where lacrimation in a few birds along with sudden death of one or two chickens occurred prior to the onset of respiratory symptoms throughout the flock. Jordan (1958) found panophthalmia in addition to respiratory symptoms, and Raggi and Armstrong (1960) reported hemorrhagic conjunctivitis in outbreaks of laryngotracheitis.

The outstanding symptoms are gasping, rales, and coughing. Many of the birds are depressed, sitting on the floor or on the roosts (Fig. 21.1). Some exhibit symptoms of gasping with the head extended and the beak open (Fig. 21.2). Wheezing or whistling sounds may be heard as the bird gasps. In these severely affected individuals coughing is frequent and often results in the expulsion of bloody mucus from the trachea. The head of the bird may be cyanotic. Many chickens will have less severe symptoms, consisting only of tracheal rales, mouth breathing, and occasional coughing. There may be lacrimation and occasionally a serous exudate from the nares. An accumulation of excessive amounts of inflammatory exudate and blood in the lumen of the larynx, and trachea, or syrinx frequently results in death by asphyxiation.

In laying flocks a variable drop in production occurs. Hinshaw (1931) observed an average drop of 12 per cent in California flocks affected with the disease. The decline began 4 days after the onset and continued until the eighteenth day. Normal production was reached again after 30 days. In colder climates and in chickens affected late in their laying year, the decline in production may be much greater. Beach (1925) observed a drop of 62 per cent in one flock.

A mild, enzootic form of the disease has been reported by Pulsford (1953, 1954), and by Simmons et al. (1954) as occurring in certain areas of Australia. More recently, Cover and Benton (1957) in the United States have recognized a mild form of the disease in broilers in which symptoms are slight coughing and rales with little or no mortality.

Course and Mortality. The average course of the disease is about 2 weeks with extremes of 1 to 4 weeks (Hinshaw, 1931a). The mortality is variable, depending upon a number of factors. Inadequate housing in winter, parasitism, and unfavorable weather conditions might influence the severity of the disease. Hinshaw et al. (1931) found an average mortality of 15 per cent in 25 outbreaks of the disease in California. The greatest mortality occurred on the eleventh day, and 85 per cent of
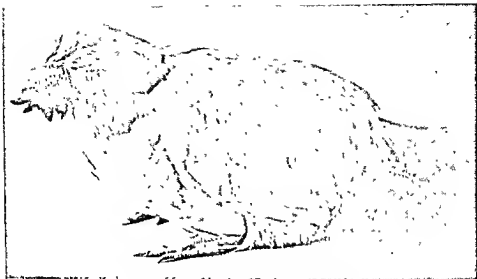
FIG. 21.1 — An advanced case of laryngotracheitis. Attitude during expiration. (Beach and Freeborn, Univ. of Calif.)

FIG. 21.2 — Laryngotracheitis. Same fowl as Figure 21.1. Attitude during inspiration.

losses were recorded 15 days after onset of the disease. Kernohan (1931b) reported a mortality of 72 per cent in one flock. Brandly and Bushnell (1934) reported a mortality of 14 per cent and 48 per cent respectively in two outbreaks. Graham *et al.* (1930) reported a subacute disease with low mortality even in artificially induced cases.

Pathology. When dead birds are presented for necropsy, the beak and mouth of the birds may be stained with blood and mucus. The mucous membrane of the trachea and larynx may be covered with a film of bright, blood-stained exudate. In some cases the lumen of the trachea may be partially or entirely occluded with exudates composed largely of blood (Fig. 21.3). A yellowish, caseous exudate, with little or no blood, may cover the mucosa

FIG. 21.3 — Hemorrhagic tracheitis in chicken caused by laryngotracheitis virus. (Hofstad and Baurfedel, Iowa State University.)

in other birds. Chickens in the early stages or with mild symptoms frequently do not have a hemorrhagic tracheitis when killed and necropsied

The microscopic pathology of laryngotracheitis has been studied in detail by Seifried (1931). Between 24 and 72 hours after inoculation there is edema and cellular infiltration of the mucosa and submucosa. Hemorrhage may separate the mucosa and submucosa. After the third day, dense cellular infiltration with a loss of epithelial structure characterizes the lesion. Inclusion bodies of the intranuclear type are found in the early stages of the disease. Seifried describes them as homogeneous structures which occupy a large part of the nucleus. The area immediately around the inclusion remains unstained and these bodies usually occur in groups of epithelial cells rather than in single isolated cells (Fig. 21.4).

The pathology in the embryonating chicken egg following inoculation of laryngotracheitis virus has been studied by Burnet (1934) and Brandly (1935). Following inoculation of the virus upon the chorio-allantoic membrane, small areas of gray thickening are visible by the third day. The lesions become larger up to the fifth or sixth day when the embryo usually dies (Fig. 21.5). There is edema of the chorio-allantoic membrane, and the allantoic fluid takes on the consistency of thick egg albumen. Embryos that live to the sixth day are stunted. Microscopically the membrane has areas of cellular proliferation and edema. Intranuclear inclusion bodies are observed in groups of ectodermal epithelial cells between the first and third days. As the lesion progresses, the epithelium undergoes necrosis and is gradually replaced by inflammatory tissue of the mesoderm.

Diagnosis. The acute, epizootic form of laryngotracheitis can be recognized frequently by the flock history, mortality record, and necropsy findings. A typical flock history is one revealing an acute, rapidly spreading, respiratory disease with symptoms of rales, mouth breathing or

gasping, fits of coughing with expulsion of bloody mucus, and some mortality. Hemorrhagic tracheitis is a typical necropsy finding It is important to choose several dead birds for necropsy rather than live, sick birds, since the latter frequently do not show the extensive tracheal lesions.

In the mild form of the disease a field diagnosis is difficult and laboratory aid is required.

Other diseases which may be confused with laryngotracheitis are infectious bronchitis and Newcastle disease. A few differences between the three diseases aid in the diagnosis. Laryngotracheitis is rarely observed in young brooder chicks, although they are fully susceptible to the virus. Of the three diseases Newcastle disease has the most adverse effect on egg production, fol-

lowed in order by infectious bronchitis and laryngotracheitis. A complete cessation of production is not unusual in outbreaks of Newcastle disease.

A greater mortality is usually experienced in the acute, epizootic form of laryngotracheitis than in outbreaks of Newcastle disease, although mortality in the latter disease can be extremely variable. As a rule, no mortality is experienced in outbreaks of infectious bronchitis in chickens over 2 months of age. The hemorrhagic tracheitis found frequently in birds dying from laryngotracheitis is not usually observed in Newcastle disease and infectious bronchitis.

Laboratory diagnoses of laryngotracheitis are made by intratracheal inoculation of susceptible and immune chicks with a

FIG. 21.4 – Tracheal mucosa from chick killed on fifth day following intratracheal inoculation. Arrow points toward a group of nuclei containing inclusion bodies characteristic of infectious laryngotracheitis. ×1,200.
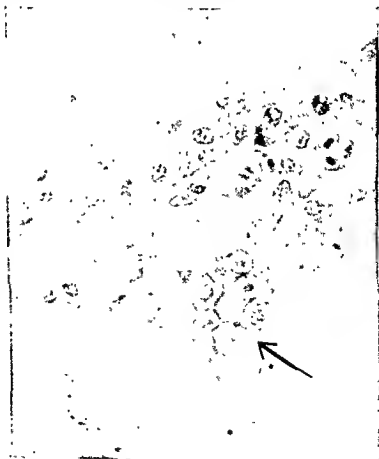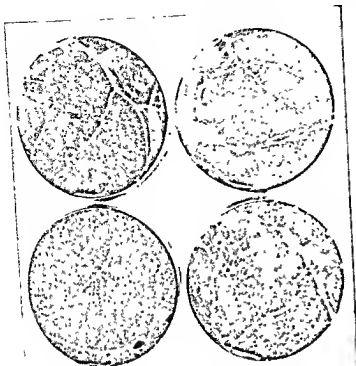
FIG. 21.5 — Lesions on chorioallantoic membrane produced by laryngotracheitis virus following inoculation into the allantoic sac. Normal membrane shown in lower left. (Hofstad and Bauriedel, Iowa State University.)

broth suspension of tracheal exudate from a suspected case, by isolation of the virus in embryonating chicken eggs, or by microscopic finding of inclusion bodies in tracheal sections. Armstrong (1959) has found smears of tracheal and conjunctival cells stained with Giemsa's stain to be a satisfactory method of examining for inclusion bodies. Woernle and Brunner (1961) and Jordan and Chubb (1962) have used the agar diffusion technique in the diagnosis of laryngotracheitis. It is possible to detect either virus antigen or antibody. In chick inoculations, consideration is given to the incubation period, the lesions produced, and the finding of inclusion bodies in tracheal sections. Inclusion bodies can be found between the first and fifth day after inoculation. The time at which inclusions are found may depend on the concentration of virus in the inoculum and the method and route of inoculation. Following intratracheal inoculation of laryngotracheitis virus by swab, inclusions were found only on the second and third days (Hanson, 1957). The inclusions may be found in tracheal sections from the original birds if presented for diagnosis in the early stages of the disease. Inclusion bodies may be demonstrated from tracheal and conjunctival cells smeared on a slide and stained with Giemsa's stain. Keller and Hebel (1962) attempted tracheal smears in 60 specimens, and 34 samples were positive for inclusions while virus was isolated 43 times. Inclusions were demonstrated 4 times when no virus was isolated. Hanson (1957) has emphasized the necessity of using Zenker's fixative with acetic acid. Pirozok et al. (1957) have described a rapid method, using a water soluble wax to eliminate dehydration and clearing processes, for the histological diagnosis of laryngotracheitis. Sevoian (1960) has used simultaneous fixing and dehydration of tissues for rapid demonstration of inclusion bodies.

A common method of making a laboratory diagnosis is to inoculate embryonating chicken eggs at 9 to 12 days of age. A broth suspension of tracheal exudate, treated with antibiotics or filtered through a suitable filter, may be inoculated upon the chorio-allantoic membrane or into the

allantoic sac. While allantoic sac inoculation is satisfactory for propagation of laryugotracheitis virus, inoculation on the dropped chorio-allantois of 12-day-old embryos is preferred for original isolation. Webster (1959) found that virus could not be isolated after the seventh day following experimental infection. Hitchner and White (1958) have observed that titration of the virus on the dropped chorio-allantoic membrane gave a two log higher titer than four other methods of inoculation, including the allantoic sac route. Laryngotracheitis virus produces areas of grayish thickening on the chorio-allantoic membrane by the third day (Fig. 21.5). Inclusion bodies can be observed between the first and third days in sections of the membrane. (See Pathology.)

Following inoculation of laryngotracheitis virus into the sinus of chickens, there develops a sinusitis with swelling, a nasal discharge, and lacrimation (Hanson, 1957). The sinus is very sensitive to the virus, and this method can be useful for diagnosis and for titration of the virus.

Burnet (1936) and Pulsford (1953, 1954) have used a serum neutralization test for studies of the epizootiology of the disease. Burnet's test makes use of the pock counting technique. The undiluted serum is mixed with 10-fold dilutions of virus and the mixture allowed to stand for one hour at room temperature. The mixture is then inoculated on the dropped chorio-allantoic membrane of 12-day-old embryos, and after 3 days' incubation the eggs are examined for pocks. A marked reduction of the pock count over the virus control is indicative of a positive serum. Hitchner et al. (1958) used the serum neutralization test and found that a 1:5 dilution of serum gave satisfactory results and found no difference between heated and unheated serum in the test; however, Hanson (1957) has found it important to heat-inactivate serum immediately after collection and before storage in the freezer to stabilize the neutralizing capacity of the

serum to be used in the serum neutralization tests. Shibley et al. (1962) found no correlation between serum-virus-neutralizing indices and resistance to challenge.

Treatment. The birds will recover rapidly as soon as an immunity develops. No medicinal treatment has been found to relieve the dyspnea of affected birds. Occasionally a veterinarian might relieve some chickens by removing the caseated casts of exudate from the larynx and upper trachea with a forceps. In outbreaks where only part of the flock is affected, it might be possible to vaccinate those groups not yet affected and render them immune before the disease spreads. Vaccination should not be carried out, however, unless a diagnosis is reasonably certain.

Prevention and control. Sound management practices have been used with success in the prevention of laryngotracheitis (Gibbs, 1933b). The introduction of the disease on the farm can be avoided by adding new stock only from clean sources, preferably as day-old chicks, by using only clean equipment, and by applying strict isolation practices.

When the disease has occurred on a farm, prevention of recurrence may be accomplished without vaccination. The recovered birds should be marketed, preferably before new stock is brought on the farm. After all chickens are gone, the house and all equipment are thoroughly cleaned and disinfected. If possible, the house, yard, and equipment used by the infected birds should be left unused for two months after disinfecting as an added assurance that the virus has been inactivated.

Prevention by vaccination is used to immunize unexposed groups of birds when the disease has just appeared in one pen on a farm, to immunize flocks on farms where the disease has occurred and where the recovered birds must be kept for breeding stock, and to immunize flocks in areas where the disease is prevalent.

Vaccination. The ability of the virus of laryngotracheitis to multiply in the mucous membrane of the cloaca was first demonstrated by Hudson and Beaudette (1932b). They found it possible to make serial passages of the virus from the cloacal mucous membrane of one bird to that of another. Later these chickens were found to be immune to intratracheal inoculation of the virus. This finding led to a practical method of immunizing flocks against laryngotracheitis. Extensive field trials with the vaccine proved its effectiveness in the prevention of laryngotracheitis (Beaudette and Hudson, 1933; Beach et al., 1934; Gibbs, 1933a, 1934).

Following vaccination the birds resist an intratracheal challenge by the ninth day (Beaudette and Hudson, 1933). During the first nine days virus can be detected in swabs from the cloaca, but the virus does not persist for longer periods (Gibbs, 1933a; Beach, 1935). That carriers did not exist after the vaccination reaction had subsided was an important consideration in using the vaccination procedure for the control of laryngotracheitis. The duration of immunity is not been determined, although results indicate that birds are protected for at least a year. Hitchner and Winterfield (1960) found chickens which had been vaccinated in the vent by the drop or brush method at 1, 4, or 8 weeks were susceptible to a challenge virus when given in the infraorbital sinus at 16 weeks of age. Revaccination by the vent method failed to give a "take" or secondary antibody response, although dropping vaccine in the eye induced a good serological response. Shibley et al. (1963) have found immunity to heterologous strains to persist for at least one year when the avirulent strain (Cover and Benton, 1957) was dropped into the conjunctival sac of 10-week-old chickens.

Dried tracheal exudate from infected chickens was originally used as the vaccine. However, egg-propagated virus was later utilized because of its greater safety,

more uniform potency, and decreased cost of production. Brandly (1936) found that as many as 36 serial transfers of the virus in embryos did not decrease its virulence or immunizing ability. It was further observed by Beaudette (1939) that egg-propagated laryngotracheitis virus, when dried and held in a vacuum under refrigeration, retained its immunizing power for as long as 421 days. Goldhaft (1961) found laryngotracheitis vaccine held for 24 years at 40° F. protected all chickens vaccinated.

In the vaccination procedure the operator exposes the mucous membrane of the upper wall of the cloaca and brushes the vaccine on the exposed mucous membrane. The brush should be dipped into the vaccine before each application. Growing chickens may be vaccinated into the bursa of Fabricius, using a blunt curved hypodermic needle, but the method is seldom used. Since the bursa undergoes atrophy during the latter part of the growing period, the method cannot be used in vaccinating adult birds. Inoculation into the bursa of Fabricius results in a larger percentage of "takes" than does the brushing method (Gibbs, 1933a; Beach et al., 1934). The best age for prophylactic vaccination of layers and breeders is between 2 and 4 months of age. Pulsford and Watts (1961) have shown that chicks from endemic areas had strong passive immunity to laryngotracheitis. This might influence the response to vaccine if vaccination is done at an early age.

Following vaccination the birds should be examined for takes. Gibbs (1934) found that the best time to do this was on the fourth or fifth day, although the reaction could be detected from the third to the eighth day. A take is evidenced by an edematous swelling of the lips of the cloaca and a catarrhal, hemorrhagic, or fibrinous inflammation of the infected mucous membrane (Fig. 21.6). When chickens are found with no visible reaction to the vaccine, they should be re-

vaccinated immediately and, if possible, segregated until they can be re-examined for takes 4 or 5 days later. It is important to revaccinate chickens with no takes, be-

cause the chickens may acquire the natural disease from the virus being eliminated from the cloaca of the vaccinated birds.

FIG. 21.6 — Taken from laryngotracheitis vaccination: (A) swelling of cloacal lips, (B) reddening of mucous membrane. (Beach and Freeborn, Univ. of Calif.)

REFERENCES

Atherton, J. G., and Anderson, W.: 1957. Propagation in tissue culture of the virus of infectious laryngotracheitis of fowls. Australian Jour. Exper. Biol. Med. Sci. 35:335.

Armstrong, W. H.: 1959. A slide smear technique for the diagnosis of laryngotracheitis. Avian Dis. 3 80.

Bakos, K., Karlsson, K. A., and Hanko, E: 1962. Eine milde form der infektiosen Laryngotracheitis (ILT) des Huhnes in Schweden. Zentralbl. Veterinarmed. 9:1.

Beach, J. R.: 1925. Infectious bronchitis of fowls. Jour. Am. Vet. Med. Assn. 68:570.

———: 1930. The virus of laryngotracheitis of fowls. Science 72.633

———: 1931a. A bacteriological study of infectious laryngotracheitis of chickens. Jour. Exper. Med. 54:801

———: 1931b. A filtrable virus the cause of infectious laryngotracheitis of chickens. Jour. Exper. Med. 54:809.

———: 1935. The survival of the virus of infectious laryngotracheitis in the bursa of Fabricius and cloaca of chickens after "intrabursal" injections. Jour. Infect. Dis. 57:155.

———, Schalm, O. W., and Lubbehusen, R. E.: 1934. Immunization against infectious laryngotracheitis of chickens by "intrabursal" injection of virus. Poultry Sci. 13.218.

Beaudette, F. R.: 1930 Infectious bronchitis. New Jersey Agr. Exper. Sta., Ann. Rep. 51.288.

———: 1939. The viability and immuning value of egg-propagated laryngotracheitis virus Jour. Am Vet. Med. Assn. 95 833

———, and Hudson, C. B.: 1935. Experiments on immunization against laryngotracheitis in fowls. Jour. Am. Vet. Med. Assn. 92 460.

———, Miller, B. R., Bevins, J. A., and Hudson, C. B.: 1948. The viability of dried viruses of avian origin. Am. Jour. Vet. Res 9(31).190.

Brandly, C. A.: 1935. Studies on infectious laryngotracheitis. The continued propagation of the virus upon the chorio-allantoic membrane of the hen's egg Jour. Infect. Dis. 57:201.

———. 1936. Studies on the egg-propagated viruses of infectious laryngotracheitis and fowl pox Jour. Am. Vet. Med. Assn. 88:587.

———, and Bushnell, L. D.: 1934. A report of some investigations of infectious laryngotracheitis. Poultry Sci. 13.212.

Burnet, F. M.: 1934. The propagation of the virus of infectious laryngotracheitis on the chorioallantoic membrane of the developing egg. Brit. Jour. Exper. Path. 15:52.

———: 1936. Immunological studies with the virus of infectious laryngotracheitis of fowls using the developing egg technique. Jour. Exper. Med. 63 685.

Chang, P. W., Yates. V. J, Dardiri, A. H., and Fry, D. E.: 1960. Some observations on the propagation of ILT in tissue culture. Avian Dis. 4.384.

Chomiak, T. W., Luginbuhl, R. E., and Helmboldt, C. F.: 1960. Tissue cultures and chicken embryo techniques for infectious laryngotracheitis studies. Avian Dis. 4.235.

Cover, M. S., and Benton, W. J.: 1957. The isolation of a viral agent from chickens showing respiratory distress Avian Dis. 1.54.

Dobson, N.: 1935. Infectious laryngotracheitis in poultry. Vet. Rec. V.N.S. 15:1467.

Gibbs, C. S.: 1931a. Infectious tracheitis. Mass. Agr. Exper. Sta., Bul. 273.

———: 1931b. Infectious laryngotracheitis carriers. Mass. Agr. Exper. Sta., Bul. 278.

———: 1932. Chronic carriers of infectious laryngotracheitis. Jour. Am. Vet. Med. Assn. 81:651.

———: 1933a. The immunology of infectious laryngotracheitis. Mass. Agr. Exper. Sta., Bul. 295.

———: 1933b. The Massachusetts plan for the eradication and control of infectious laryngotracheitis. Jour. Am. Vet. Med. Assn. 83 214.

———: 1954. Infectious laryngotracheitis vaccination. Mass. Agr. Exper. Sta., Bul. 511.

Goldhaft, T. M.: 1961. Viability of pox and laryngotracheitis vaccines. Avian Dis. 5:196.

Graham, R., Thorp, F., Jr., and James, W. A.: 1930. Subacute or chronic infectious avian laryngotracheitis. Jour. Infect. Dis. 47:87.

———, Thorp, F., Jr., and James, W. A.: 1931. A filtrable virus-like agent in avian laryngotracheitis. Jour. Am. Vet. Med. Assn. 78:506.

Gwatkin, R.: 1925. Some notes on avian diphtheria (chicken pox). Report of the Ontario Veterinary College for 1924, p. 54.

Hanson, R. P.: 1957. Personal communication.

Hinshaw, W. R.: 1931a. A survey of infectious laryngotracheitis of fowls. Calif. Agr. Exper. Sta., Bul. 520.

———: 1931b. Infectious laryngotracheitis of fowls. Vet. Med. 26:324.

Jones, E. E., and Grayball, H. W.: 1951. A study of mortality and egg production in flocks affected with infectious laryngotracheitis. Poultry Sci. 10:575.

Hitchner, S. B., Shea, C. A., and White, G. P.: 1958. Studies on a serum neutralization test for the diagnosis of laryngotracheitis in chickens. Avian Dis. 2:258.

——— and White, P. G.: 1958. The correlation of embryo and bird infectivity with five strains of laryngotracheitis virus. Poultry Sci. 37.684.

———, and Winterfield, R. W.: 1960. Revaccination procedures for infectious laryngotracheitis. Avian Dis. 4:291.

Hofstad, M. S., and Yoder, H. W., Jr.: 1963 Inactivation rates of some lyophilized poultry viruses at 37° and 3° C. Avian Dis. 7:170.

Holmes, F. O.: 1948. In Bergey's Manual of Determinative Bacteriology, Sixth Ed. Williams and Wilkins Co., Baltimore. P. 1274.

Hudson, C. B., and Beaudette, F. R.: 1932a. The susceptibility of pheasants and a pheasant-bantam cross to the virus of infectious bronchitis. Cornell Vet. 22.70.

——— and Beaudette, F. R.: 1932b. Infection of the cloaca with the virus of infectious bronchitis. Science 76:34.

Jordan, F. T. W.: 1958. Some observations on infectious laryngotracheitis in the diagnosis of infectious laryngotracheitis.

——— and Chubb, R. C.: 1962. The agar gel diffusion technique in the diagnosis of infectious laryngotracheitis (ILT) and its differentiation from fowl pox. Res. Vet. Sci. 3(3):245.

Keller, K., and Hebel, P.: 1962. Diagnostico de las inclusiones de laringotraqueitis infecciosa en frotis y cortes histológicos. Zooiatria (Chile) 4(1):1.

Kernohan, G.: 1930. Infectious bronchitis in fowls. Calif. Agr. Exper. Sta., Bul. 494.

———: 1931a. Infectious laryngotracheitis in pheasants. Jour. Am. Vet. Med. Assn. 78:553.

———: 1931b. Infectious laryngotracheitis of fowls. Jour. Am. Vet. Med. Assn. 78:196.

Kingsbury, F. W., and Jungherr, E. L.: 1957. Indirect transmission of infectious laryngotracheitis in chickens. (Abst.) Poultry Sci. 36:1153.

Komarov, A., and Beaudette, F. R.: 1932. Carriers of infectious bronchitis. Poultry Sci. 11:335.

Magnusson, H.: 1930. En ny bonsjukdom (Inf. laryngotrakeit). Skand. Vet.-Tidskr. 30.629.

Marek, K.: 1948. Zakázny laryngotracheitis kur. hledyczna Weterynaryjna 4:767.

May, H. G., and Tittsler, R. P.: 1925. Tracheo-laryngitis in poultry. Jour. Am. Vet. Med. Assn. 67:229.

Pirozok, R. P., Helmboldt, C. F., and Jungherr, E. L.: 1957. A rapid histological technique for the diagnosis of infectious avian laryngotracheitis. Jour. Am. Vet. Med. Assn. 130:406.

Pulsford, M. F.: 1953. Possible P-Q type variation in the strains of infectious laryngotracheitis virus. Nature, London, 172:1193.

———: 1954. Variation in the virus of infectious laryngotracheitis and its epizootiogical implications. 10th World's Poultry Cong. p. 242.

———: 1960. The growth of three strains of infectious laryngotracheitis virus of fowls in tissue culture. Australian Jour. Exper. Biol. Med. Sci. 38:153.

——— and Watts, P. S.: 1961. Natural passive resistance of chickens from endemic areas to infection by infectious laryngotracheitis. Australian Vet. Jour. 37:314.

Raggi, L. G., and Armstrong, W. H.: 1960. Conjunctivitis of chickens caused by a typical infectious laryngotracheitis virus. Avian Dis. 4:272.

Satriano, S. F., Luginbuhl, R. E., and Jungherr, E. L.: 1957. Isolation of infectious laryngotracheitis virus from lacrimal fluid of chick. (Abst.) Poultry Sci. 36:1155.

Schalm, O. W., and Beach, J. R.: 1935. The resistance of the virus of infectious laryngotracheitis to certain physical and chemical factors. Jour. Infect. Dis. 56.210.

Seddon, H. R.: 1936. Infectivity experiments with the virus of laryngotracheitis of fowls. Australian Vet. Jour. 12:15.

———, and Hart, L.: 1935. The occurrence of infectious laryngotracheitis in chickens. Jour. Exper. Med. Australian Vet. Jour. 11:212.

Seifried, O.: 1931. Histopathology of infectious laryngotracheitis in chickens. Jour. Exper. Med. 54:817.

Sevoian, M.: 1960. A quick method for diagnosis of avian pox and ILT. Avian Dis. 4.474.

Shibley, G. P., Luginbuhl, R. E., and Helmboldt, C. F.: 1962. A study of ILT virus. I. Comparison of serologic and immunologic properties. Avian Dis. 6 59.

Shibley, G. P., Lugnbuhl, R. E., and Helmboldt, C. F.: 1963. A study of infectious laryngotracheitis virus. II. Duration and degree of immunity induced by conjunctival vaccination. Avian Dis. 7:184.

Simmons, G. C., Ryley, J. W., and Macdonald, V. M.: 1954. Infectious laryngotracheitis of poultry in Queensland. Australian Vet. Jour. 30:129.

Special Committee on Poultry Diseases: 1931. Jour. Am. Vet. Med. Assn. 79.522.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflugelzucht. Ferdinand Enke, Stuttgart P. 262.

Watrach, A. M., Vatter, A. E., Hanson, L. E., Watrach, M. A., and Rhoades, H. E.: 1959. Electron microscopic studies of the virus of avian ILT. Am. Jour. Vet. Res. 20.537.

Webster, R G.: 1959. Studies on infectious laryngotracheitis in New Zealand. N.Z. Vet. Jour. 7.67.

Woernle, H., and Brunner, A: 1961. Präzipitationstest zur diagnose der Infektiösen Laryngotracheitis des Huhnes. Tierarztl. Umsch. 16:245.

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22

Newcastle Disease

An infectious, highly contagious and de-
structive malady, Newcastle disease (ND)
attacks chiefly chickens and turkeys.
Various other birds as well as certain mam-
mals, including man, may also contract the
disease. ND is caused by a virus which is
classified as a myxovirus and resembles the
viruses of influenza and mumps in certain
properties.

Occurrence

First recognized in the middle 1920's,
Newcastle disease has become a major
menace to the world's poultry industry.
Kraneveld, in 1926, reported a highly dif-
fusible and fatal infection of poultry prev-
alent in the Dutch East Indies, and in the
same year, Doyle saw the disease near New-
castle-on-Tyne, England. Doyle named it
after the locality and demonstrated it to be
distinct from the dreaded fowl plague or
fowl pest.

The outbreak in England was promptly
stamped out by quarantine of the premises,

slaughter of infected and exposed flocks,
and disinfection of contaminated equip-
ment and premises. Eradication of the
Oriental outbreaks was not attempted, and
the malady is now present in every poultry
producing area of the world.

During the decade following recognition
of what is now generally termed ND, the
malady was reported also from India, the
Philippine Islands, Korea, Japan, Australia,
Ceylon, and Kenya. Subsequently, it ap-
peared in Palestine, Syria, and the middle
Congo, and, with World War II, it spread
to Europe by way of Sicily and Italy. It was
first identified in the United States in 1944
(Brandly et al., 1944, 1946a; Beach, 1944)
although, according to Beach, it apparently
had been present in California as early as
1935. Beaudette and Hudson (1956) refer
to isolation of ND virus (NDV) in Illinois
and New Jersey in 1938.

In the initial appearance of the malady
in these and later foci, its identity with
Newcastle disease of Doyle (1927) was

[633]

seldom recognized, and at least 15 synonyms, e. g., pseudo-fowl pest, *Pseudovogelpest, atypische Geflügelpest*, pseudo-poultry plague, avian pest, avian distemper, *Ranikhet*, avian pneumoencephalitis, have been recorded (Brandly *et al.*, 1946a). By 1918, the malady had spread throughout this country and to Hawaii and Canada, and, by 1951, it had become disseminated widely in Europe and in various parts of Africa. It was also recognized in Madagascar, Mexico, and Central and South America (Beaudette, 1943, 1949, 1950, 1951). Following later reports of its proved occurrence in China (Ma *et al.*, 1947) and Indo-China, Liao (1951) observed that a disease known as chicken pest, which may have been ND, had been known in China for many generations. A mild outbreak in Japan (Kawashima *et al.*, 1953), the occurrence of mild strains in France (Lissot, 1956), persistence of milder forms in England (Reid, 1955), and reintroduction of a fatal form in Kenya (Scott *et al.*, 1956) have been recorded more recently.

In the absence of land or sanitary barriers between adjacent areas or across water and air routes, the eventual wide diffusion of ND was inevitable. First reports of the disease during the early 1960's including several from South American and African countries (Monteverde *et al.*, 1962; Kaschula, 1961a) reaffirmed its high diffusibility and current prevalence in all major poultry producing areas. Reappearances of frequently severe epidemics of ND also continue to be reported: Kaschula, 1961a in Africa; Brion and Fontaine, 1960 in France; Divo, 1961 in Venezuela.

The early lack of definite diagnostic procedures together with the differences in certain clinico-pathologic features manifested on the appearance of ND in new areas have frequently delayed its recognition and the eventual development of more effective control measures.

Absence of the virus from the blood stream of birds was a confusing feature of certain earlier outbreaks in the Orient. The considerable delay in suspecting and

identifying ND after it invaded the U.S.A. can be chiefly ascribed to features previously not ascribed to the malady, namely, relatively low mortality and predominance of nervous and respiratory manifestations. With recent substantial improvements of immunologic and other diagnostic methods, long delays and failures in recognition of the milder as well as more severe outbreaks should be largely avoidable.

Doyle (1935) recognized the shortcomings of the eponymic term, Newcastle disease, but, in view of prevalent usage rather than priority, believed its use warranted until further clarification could be accomplished. The proposal that the task of selecting a more suitable name be referred to a duly constituted body on nomenclature (Brandly, 1947) may still warrant consideration.

IMPORTANCE

Newcastle disease represents a serious economic challenge to all segments of the poultry industry. The proclivity of NDV to attack not only poultry and birds, but man and other mammals as well, magnifies its potential as well as actual threat. The high resistance of NDV to adverse environment and the facility with which it may spread, especially by air, further contribute to the gravity of the problem.

The high mortality described in the initial reports (Kraneveld, 1926; Doyle, 1927) has characterized many early as well as later outbreaks: Crawford in Ceylon, 1930; Sahai in India, 1937; Albiston and Gorrie in Australia, 1942; Coronel in the Philippines, 1947; Kaschula in Africa, 1961b; Reid in Great Britain, 1961. More recent outbreaks in Mexico and an exotic introduction in California were likewise highly fatal (Report of Chief of Bureau of Animal Industry, 1950). Absence of mortality among adult chickens has been reported from various parts of the world, e.g., Dobson from England (1939), Beach (1942), Bankowski (1961), and others from the United States, and Komarov from Palestine 1947), while, following the 1947 epizootic in England, a low or subclinical

form of the disease was recognized except for occasional mild signs in chicks (Anonymous, 1948). Highly variable mortalities from ND have been encountered in the United States (Beach, 1942; Barber, 1947; Goldhaft and Wernicoff, 1948). The latter workers saw both low (10 per cent) and high (90 per cent) fatalities in adjacent poultry flocks in New Jersey. Binns *et al.* (1949) reported a relatively fatal epizootic in Utah with losses up to 86.5 per cent and an average mortality of 48.6 per cent in eight flocks, totaling 13,639 birds of various ages.

The losses from the nonfatal outbreaks, while less spectacular, may be equal to or greater than those where the death loss is high. Crippling as well as impaired growth and feed utilization among surviving birds follow outbreaks of ND. An increase of two or more weeks in finishing surviving broiler chickens for the market is not uncommon. The mortality among turkey poults is usually not as great as among chicks but may reach 15 to 20 per cent of a brood. The greatest loss among layers frequently results from reduced production and impaired eggshell and albumen quality. ND also reduces the fertility and hatchability of eggs. The virus has produced teratogenic defects and early mortality among experimentally infected embryos (Blattner and Williamson, 1951; Williamson, *et al.*, 1956).

A survey of ND prevalence in an intensive poultry farming area in 1947 (Byerly, 1948) showed a 30 per cent infection rate. Soon after ND became widespread in the United States, the livestock sanitary officials of twenty-seven states listed ND as their foremost problem in poultry production (Linn, 1948). An additional loss exacted by ND comes from restrictions upon exports of eggs, both hatching and other, and of baby chicks, of breeding and other stock, and of dressed poultry. Risk of NDV contamination of various vaccines for poultry and other animals reduces the demand for such exports to other countries.

In the United States alone, about 2.69 billion chickens and 99 million turkeys were hatched in 1962. An estimated 1 per cent mortality from ND would represent 27 million chickens; a comparable heavy loss from the other effects of the disease would raise the annual total to 35 million dollars. An additional toll in the struggle against ND as it prevails in the United States is the cost of vaccination against it. A conservative estimate of the annual cost of vaccinating one billion birds against ND is 15 million dollars. This would raise the estimated yearly toll of ND in the United States to more than 50 million dollars. On an equal basis, the annual tribute exacted from the world's poultry industry would conservatively double this figure.

The grouping and designation, as practiced in England (1936 Fowl Pest order) and European countries, of Newcastle disease and fowl plague or pest while perhaps having some merit from the regulatory control aspect, tend to becloud the problem of specific diagnosis and of prophylactic intervention. Furthermore, on the matter of obtaining reliable data on occurrence, prevalence, and incidence of ND and diseases resembling it becomes more complicated.

Newcastle disease has been recognized in man as almost entirely a localized eye infection yet pulmonary and generalized infections have been reported. While infection of one person from another has not been recognized, the possibility that ND may become a more serious public health problem must be recognized (Burnet, 1943; Brandly, 1950, 1951; Cunningham, 1952; Hanson and Brandly, 1958).

NATURE OF THE VIRUS

The agent of Newcastle disease was recovered in 1926 by T. M. Doyle (1927) from diseased birds, and shown to be filterable and distinguishable antigenically from the virus of fowl plague. Burnet and Ferry (1934), by filtration data, estimated the size of the viral particles to be 80 to 120 millimicrons. On the basis of electron micrographs, Bang (1948) later calculated the

diameter to be 112 mμ. The present and much more detailed picture of the morphology of Newcastle disease virus is based upon several methods of purification and on examination by negative staining (Waterson, 1964; Rott and Schäfer, 1961; Schäfer, 1963). The virion, or mature virus unit which varies in size from 150 to 200 mμ (Waterson and Cruickshank, 1963) consists of an envelope and an internal component. The either-sensitive and osmotically deformable envelope (Bang, 1946) has a pattern of projections or spikes (80 A° long) and contains the antigenic components that stimulate the host to produce hemagglutinin-inhibiting and virus-neutralizing antibodies (Rott, 1964). The internal component, or nucleocapsid, also known as the G-antigen or NP-antigen, consists of a long and much coiled tube which has a diameter of 180 Angstroms (Schäfer and Rott, 1959). The protein structural units of the tube are arranged in a helix around the central hollow axis. Within them and determining the entire configuration is ribonucleic acid.

The Newcastle disease virion is morphologically similar to but antigenically distinct from other viruses of the parainfluenza group and, like them, it is inactivated by hydroxylamine (Rott and Schäfer, 1962). With the exception of myxovirus Yucaipa which was isolated in California and produces a mild respiratory disease in chickens (Bankowski and Corstvet 1961; Dinter et al., 1964) and fowl parainfluenza 2 of Ruckle-Easters (Waterson, 1964), the other parainfluenza viruses produce disease in mammals. Among them are parinfluenza of cattle, respiratory syncytial virus and mumps virus of man, and the antigenically related group of measles, distemper, and rinderpest (Chanock and Coates, 1964).

The true influenza viruses have a smaller virion, 80 to 120 mμ, and a smaller nucleocapsid, 90 Angstroms in diameter. Influenza viruses affecting birds include fowl plague, virus N, Czechoslovakian and British duck influenzas, and tern disease (Dinter, 1964). The avian influenzas share an antigenic component with influenza A of man, swine, and horses. Influenza B, which affects man, is antigenically distinct.

Newcastle disease virus possesses a number of biological and physical characteristics by which it may be distinguished from other myxoviruses and by which strains of NDV may be distinguished from each other. Among these properties are the ability to agglutinate and to lyse erythrocytes, to induce toxic changes without accompanying multiplication, to enter cells and to replicate, to infect and to incite various signs and lesions in certain hosts and to cause the death of some hosts, to be specifically neutralized by antibodies, and to be inactivated by selected agents under defined conditions. If the procedures used are properly standardized and controlled, all the actions can be quantitated and the results obtained can be repeated with considerable degree of exactness.

From the standpoint of economics, the most important property of NDV is its ability to produce disease and death of chickens: a property in which the strains of virus differ markedly. All strains may be grouped for convenience into three classes of differing virulence for chickens: velogenic, mesogenic, and lentogenic (Hanson and Brandly, 1955). Irrespective of the route of exposure, chickens receiving velogenic strains develop severe disease which often terminates in death. Embryos, after receiving a minimal lethal dose, die within 50 hours. When introduced by peripheral routes mesogenic strains usually produce a mild disease which rarely results in death of the chicken. If the virus is introduced directly into the central nervous system, severe disease and death result. Chicken embryos are killed within 50 to 60 hours after receiving a minimum lethal dose. Lentogenic strains produce a mild or inapparent disease irrespective of method of exposure. If the lentogenic virus is introduced directly into the central nervous system, it does not multiply and it does not result in death of the bird. Embryos are

killed 100 hours after receiving a minimal lethal dose.

Foremost among the biological properties of NDV is its ability to adsorb to the surface of red cells and to induce their aggregation. Hemagglutination of NDV was first described by Burnet (1942) who also found this action to be inhibited by specific antiserum. The hemagglutinin is structurally identified with projections on the envelope of the virus (Rott, 1964) and it has been chemically determined to be the enzyme, neuraminidase. Hemagglutination may be measured by the sedimentation rate of agglutinated red cells (Hirst and Pickels, 1942) or by the pattern which the aggregated cells form on a curved glass surface (Salk, 1944). The latter means of detection has been found to be the most practical method of quantitating the hemagglutinating activity of the virus. The process of hemagglutination consists of two stages: the attachment of the virus to the receptor substance on the cell surface (agglutination) and the destruction of the receptor substance by the enzyme neuraminidase (Ackerman, 1964). The second stage (Sagik and Levine, 1957; McCollum and Brandly, 1955a) is associated with the release of the virus from the surface of the cell (elution). While erythrocytes of all amphibia, reptiles, and birds are agglutinated to some degree by NDV (Clark and Nagler, 1943), some mammalian erythrocytes are inagglutinable. Man, mouse, and guinea pig erythrocytes are agglutinated by all strains of NDV; those of cattle, goat, sheep, swine, and horses are agglutinated by some but not all strains of NDV (Winslow et al., 1950b). The erythrocytes of some individual cattle are agglutinated by one Newcastle strain while cells of another individual are not agglutinated by the same strain. The sensitivity of all cells to agglutination, but particularly so variably reactive cells as those of cattle, is dependent upon ionic concentration of the salts and the pH of the suspending solution. Erythrocytes are not the only type of cell to which the virus absorbs and which may be agglutinated as a result of this absorption. Chu (1953) demonstrated that sperm cells may be agglutinated by the action of Newcastle disease virus. The absorption of NDV to brain cells may be demonstrated by the reduction it induces in the ability of the virus preparation to subsequently agglutinate avian erythrocytes (Piraino and Hanson, 1960).

Approximately 100,000 virus infective units equal an hemagglutinating unit. The number of viral particles in a preparation may be approximated on the basis of its hemagglutinin activity. However, the rate of inactivation of the hemagglutinating activity of the virus is not necessarily proportional to the rate of the inactivation of the infectivity of the virus preparation (Hanson et al., 1949; Tolba and Eskarous, 1962). Some strains of Newcastle disease virus which lose their hemagglutinative activity on treatment at 56° C. within 5 minutes, remain capable of infecting embryonated eggs or other suitable hosts even after a further period of 25 minutes of inactivation. Other strains of NDV retain their ability to hemagglutinate after treatment at 56° C. for 180 to 240 minutes although their ability to produce infection was lost within the first 90 minutes. In this respect, NDV differs from the influenza viruses, all of which lose their ability to infect embryonated eggs before they lose their ability to hemagglutinate. The difference in the rate at which the hemagglutinin is destroyed by heat is a characteristic by which NDV strains may be differentiated.

Like other parainfluenzas, NDV possesses a hemolysin. The virus is capable of lysing those erythrocytes that it can agglutinate (Kilham, 1949; Burnet, 1950; Burnet and Lind, 1950). The hemolytic activity of virus preparations is enhanced by freezing and thawing, by dialysis, by sonic vibration, and by osmotic shock (Granoff and Henle 1954; McCollum and Brandly, 1955b). The salt concentration and pH of the suspending solution and the temperature at which the reaction takes place are important (Granoff and Henle, 1954). Picken (1964)

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638   C. A. BRANDLY and R. P. HANSON

has suggested that the hemolytic capability of NDV can be separated from its hemagglutinative activity by chemical procedures.

The ability of the viral preparation to provoke a pathologic change in a host system in the absence of multiplication has been designated toxicity. Burnet (1942) first reported that NDV possesses this capability. The toxic response is elicited following intracerebral, intranasal, and intravenous administration of the virus in mice and intravenous injection of rabbits. When introduced into the brain of a mouse, the virus incites central nervous system disturbance with paralysis within two days and death in three days (Upton et al., 1953; Groupe and Dougherty, 1956). Following nasal instillation, it may induce pneumonia and death of mice (Ginsberg, 1951), and following intravenous injection, it may cause lymphocytopenia (Evans and Melnick, 1950) and fever (French, 1952) of rabbits. Toxic capability of the virus is rapidly reduced by dilution and readily destroyed by treatments that destroy infectivity. Following inoculation of the virus into the brain of a mouse, it is absorbed by and penetrates the cells. The virus may even initiate a partial replication process as has been suggested by Cairns (1951). However, infective virus is not produced. The toxicity of NDV for mice following inoculation by any route varies among strains (Hanson et al., 1951; Upton et al., 1953). Those strains that have a high degree of neurotoxicity for mice are also highly pathogenic for chickens. No relationship has been found between toxicity measured in other ways and pathogenicity for chickens.

Replication of NDV has been studied in cell culture systems. The virion, on reaching a cell surface, presumably attaches by processes that are involved in agglutination of erythrocytes. The virion then passes through the cell wall probably by viropexis (Silverstein and Marcus, 1964). At this point the virion loses its integrity, the envelope disappears, and it is supposed that the nucleoprotein core is released as a long filament (Fig. 22.1). Infective virus cannot be found for the next three hours. During the eclipse period, NDV specific antigen can be demonstrated by complement fixation and by fluorescent antibody reactions. First detected is the NP antigen in the cytoplasm near the nucleus (Rott, 1964). Then the HA antigen and neuraminidase can be found throughout the cytoplasm. The specific antigens accumulate and structures identical with the mature virion appear just within the cell membrane 3 to 4 hours after infection (Wheelock, 1963; Kingsbury, 1962). Sometimes these particles pack the microvilli. If the cell is destroyed by sonic disruption, its particles are found to be infectious. In the usual course of events the virions are liberated as small parts of the cell membrane, much as microvilli are sloughed (Bang, 1952). Liberation of virus starts four hours after infection and can continue for another four hours without destruction of vital processes of the infected cell. An excess of virus specific substances, external NP antigen, viromicrosomes, and released HA are produced that are not incorporated into the infective virus particles, the virions (Rott, 1964).

There is some evidence that the virion may be produced either at the cell surface and incorporate cell membrane substance, or within the cytoplasm and incorporate endoplasmic reticulum substance (Rott and Schäfer, 1962). The first class of particles is sensitive to hydroxylamine and is usually avirulent for chickens. The second class is resistant to hydroxylamine treatment and is usually virulent for chickens.

NDV interferes with the multiplication and pathologic expression of certain other viruses and, in turn, it is interfered with by certain viruses (Raggi et al., 1963; Chanock, 1955; Morimoto et al., 1962). Mixed infection in chickens can result in aborted disease and immunologically reduced response (Hanson et al., 1956). In cell cultures, interference has been utilized in the development of procedures to detect noncytopathic viruses (Shimizu et al., 1964). Mixed infections of NDV strains and of
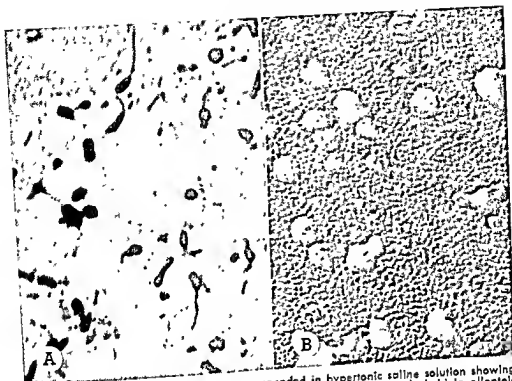
FIG. 22.1 —(A) Newcastle disease virus suspended in hypertonic saline solution showing sperm-like or filamentous form. (B) Newcastle disease virus previously held in allantoic fluid showing roughly spherical forms. Shadow cast. (F. B. Bang.)

ND and influenza viruses sometimes result in production of combined forms, a kind of recombinant (Granoff and Hirst, 1954; Granoff, 1962).

Newcastle disease virus is capable of producing changes in many tissue culture systems. These changes are of two primary types: necrosis of the cell and alteration in form or physiology of the cell. The alterations may be characterized by increased permeability to dyes or by formation of giant cells. Many types of primary cells, particularly of chicken embryo origin (Rubin et al., 1957; Morehouse et al., 1963), and certain cell lines of mammalian origin are readily infected by NDV (Brandt, 1961; Bankowski, 1964; Gelenczei and Bordt, 1960). On cell monolayers, many strains of NDV induce the formation of plaques (Granoff, 1964; Schloer, 1964). In these circumscribed areas of a sheet of cells, the virus has multiplied and destroyed or altered the cells in such a manner that an area of change or plaque is evident to the naked eye. The plaques produced by NDV can be grouped into several size classes, ranging from 0.5 to 2.4 mm. in diameter. The degree of the cell destruction within these plaques varies in such a way that most plaques can be described as clear if most or almost all of the cells are destroyed, or turbid if only a few of the cells have been destroyed. Some strains of the virus produce plaques with sharp margins and other strains produce plaques with a faint margin. In the latter instance, it is difficult to distinguish the boundary between the plaque and the monolayer. In the red plaques first described by Thiry (1963), there is no evidence of cell destruction. The plaque is apparent because of change in ability of infected cells to absorb dye. Syncytial formation is sometimes evident in red plaques (Schloer, 1961). Depending on temperature and nature of the monolayer, plaques develop within 2 to 4 days fol-

lowing inoculation of the virus. Since the size and clarity of a plaque increase with time, the description of a plaque must always refer to its age.

Velogenic and mesogenic strains are cytopathic and produce plaques; lentogenic strains are sometimes cytopathic but appear to be unable to produce plaques in chicken embryo fibroblasts and in HeLa cells. With these exceptions, most laboratory strains of NDV as well as new isolates contain more than one type of plaque and, sometimes, as many as five types differing in size, margination, degree of cell destruction, or type of cell alteration. It is possible to differentiate strains according to the types of plaques that they produce. It is possible to carry them as pure lines which will continue to produce under suitable conditions of serial transfer plaques that are similar to the one that was initially picked (Baron, 1964; Schloer, 1964).

All strains of NDV induce infection of chicken embryos which lead, with few exceptions, to death of the embryo. The lentogenic strains may fail to induce death of the embryo when antibodies are present in the yolk. Velogenic and mesogenic strains produce such rapid infection that the yolk antibody is incapable of modifying it. The route of infection, the temperature at which the incubated egg is held, the age of the embryo, and the quantity of virus introduced modify the infectious process. Infection is more rapidly fatal when the inoculated embryos are young, when the quantity of virus introduced is large, and when incubation is above, as contrasted to below, 37° C. (Sinha, 1958). Yolk sac and intravenous inoculation result in more rapid fatalities than do allantoic and chorioallantoic membrane inoculation (Hanson et al., 1947). The route of the infection modifies the order in which tissues become infected and the rate at which the virus titer increases. High titers of virus are found in the extra-embryonic fluids from 24 to 48 hours before the death of the embryo. The differences in time vary among strains. However, development

of high titers in the lung and spleen of the embryo itself precede death by only 2 to 6 hours. Presumably, the presence of virus in these tissues is associated with changes that result in death. Consequently, the rapidity with which these sites in the embryo are reached by the virus following its introduction probably determines the length of the period between infection and death. A measure of the virulence of a strain of NDV for the chicken embryo can be based on the number of hours that elapse between introduction of the minimal infective dose and death of the embryo (Hanson and Brandly, 1955). Lentogenic strains take over 100 hours; mesogenic and velogenic strains, 50 hours or less. The gross lesions induced by ND in the embryo include petechial hemorrhage on the skin of the embryo over the extremities and, with some strains, particularly over the cranial area (Bang, 1964).

The pathogenicity of NDV for chickens is determined largely by the strain of the virus, but also in part by the dose, the route of administration, the age of the chicken, and certain environmental conditions (Sinha et al., 1952). The younger the chicken, the more virulent is the virus. Breed or genetic stock of chicken has only a slight effect upon susceptibility to the virus. At high ambient temperatures, chickens appear to be more susceptible and to more frequently develop neurologic signs (Sinha, 1957). Some of the velogenic strains are capable of producing death following the introduction of only a few infective particles; other strains require a million or more infective units to induce death; some strains are nonfatal even in enormous doses. The route of infection modifies the action of the pathogenic process. Natural routes of infection, such as intranasal, oral, and ocular, including aerosol forms, are most frequently associated with development of respiratory disease. Intramuscular, intravenous, and intracerebral administrations usually enhance the neurovirulence of the virus.

A sequence of events following introduc-

tion of NDV into the chicken is initiated by multiplication of the virus at the site of introduction (Asdell and Hanson, 1960). There follows liberation of virus into the blood stream, a second cycle of multiplication in visceral organs, a second release into the blood stream, and passage into the central nervous system in some instances. Signs of disease and liberation of virus into the environment are associated with the second release of the virus into the blood stream. The course of the disease is determined by the defense mechanisms that come into play at this time. The pathogenicity of NDV for chickens can be measured by the ability of the virus to produce death, to produce central nervous system signs such as paralysis, tremor, and torticollis, and to produce respiratory signs which may be marked or so slight as to become evident only upon a disturbance of the birds. Degree of pathogenicity is expressed by the effect of the virus upon the ability of the bird to lay and to maintain weight gains. The virulence of NDV for susceptible chickens presents a complete continuum from very rapid fatal infection to an inapparent disease.

All NDV strains are capable of provoking an antibody response in chickens, rabbits, and in other species into which they are introduced. Newcastle disease virus has several antigens. The antigen that induces the virus neutralizing antibody and the hemagglutinating antigen are associated with the envelope of the virus; the NP antigen or soluble antigen is associated with the nucleoprotein portion of the virus. The NP antigen is detected in the complement-fixation test and in the gel-diffusion test. The hemagglutinating antigen, the virus neutralizing antigen, and the NP antigen form distinguishable lines in the gel-diffusion test (Rott, 1964). The ability of the virus to fatally infect embryonated eggs and to infect cells in culture is inhibited by the virus-neutralizing antibody. The virus also induces the production of protective antibodies in the chicken which enables it to resist reinfection. The various

serologic procedures do not necessarily detect the same antibody and antigenic reactivities. When the hemagglutinating antigen is destroyed by ether, the preparation no longer agglutinates erythrocytes and one of the lines in the gel-diffusion test is eliminated (Coleman, 1959). The difference between the virus neutralizing antibody, the hemagglutination-inhibiting antibody, and the refractivity of chickens to challenge become apparent on a temporal scale. In the serum of convalescent chickens, antibody as measured in these ways appears to increase and decrease in an independent manner (Coleman, 1959). Hemagglutination-inhibiting antibodies will become undetectable while virus-neutralizing antibody is still very high. Refractivity of chickens to reinfection often cannot be related to a circulating antibody. Antigenic differences exist among NDV strains. Strains can be distinguished on the basis of the gel-diffusion test, the hemagglutination-inhibition test, and the virus-neutralization test. These are differences that are both qualitative and quantitative in nature (Schloer, 1964).

The stability of NDV can be measured on the basis of alterations in the ability of the virus to infect, to agglutinate cells, and to induce an immunogenic response. These abilities can be destroyed at varying rates by such physical and chemical treatments as heat, light, ultra-violet, X-ray, oxidation processes, pH changes, and cresolic compounds. The rate at which the reactivity of the virus is destroyed varies with the strain of virus. It is also dependent upon the time of exposure to treatment, the quantity of virus which is initially exposed, the nature of the suspending medium, and by interactions among treatment variables. The sensitivity of the virus to thermal changes has been studied in greater detail than has any other environmental factor (Foster and Thompson, 1957; Hanson et al., 1949). All activity of the virus is destroyed at 100° C.—within a minute. At 56° C. the destruction of infectivity, hemagglutinative activity and immunogen-

lowing inoculation of the virus. Since the size and clarity of a plaque increase with time, the description of a plaque must always refer to its age.

Velogenic and mesogenic strains are cytopathic and produce plaques; lentogenic strains are sometimes cytopathic but appear to be unable to produce plaques in chicken embryo fibroblasts and in HeLa cells. With these exceptions, most laboratory strains of NDV as well as new isolates contain more than one type of plaque and, sometimes, as many as five types differing in size, margination, degree of cell destruction, or type of cell alteration. It is possible to differentiate strains according to the types of plaques that they produce. It is possible to carry them as pure lines which will continue to produce under suitable conditions of serial transfer plaques that are similar to the one that was initially picked (Baron, 1964; Schloer, 1964).

All strains of NDV induce infection of chicken embryos which lead, with few exceptions, to death of the embryo. The lentogenic strains may fail to induce death of the embryo when antibodies are present in the yolk. Velogenic and mesogenic strains produce such rapid infection that the yolk antibody is incapable of modifying it. The route of infection, the temperature at which the incubated egg is held, the age of the embryo, and the quantity of virus introduced modify the infectious process. Infection is more rapidly fatal when the inoculated embryos are young, when the quantity of virus introduced is large, and when incubation is above, as contrasted to below, 37° C. (Sinha, 1958). Yolk sac and intravenous inoculation result in more fatal fatalities than do allantoic and chorioallantoic membrane inoculation (Hanson et al., 1947). The route of the infection modifies the order in which tissues become infected and the rate at which the virus titer increases. High titers of virus are found in the extra-embryonic fluids from 24 to 48 hours before the death of the embryo. The differences in time vary among strains. However, development

of high titers in the lung and spleen of the embryo itself precede death by only 2 to 6 hours. Presumably, the presence of virus in these tissues is associated with changes that result in death. Consequently, the rapidity with which these sites in the embryo are reached by the virus following its introduction probably determines the length of the period between infection and death. A measure of the virulence of a strain of NDV for the chicken embryo can be based on the number of hours that elapse between introduction of the minimal infective dose and death of the embryo (Hanson and Brandly, 1955). Lentogenic strains take over 100 hours; mesogenic and velogenic strains, 50 hours or less. The gross lesions induced by ND in the embryo include petechial hemorrhage on the skin of the embryo over the extremities and, with some strains, particularly over the cranial area (Bang, 1964).

The pathogenicity of NDV for chickens is determined largely by the strain of the virus, but also in part by the dose, the route of administration, the age of the chicken, and certain environmental conditions (Sinha et al., 1952). The younger the chicken, the more virulent is the virus. Breed or genetic stock of chicken has only a slight effect upon susceptibility to the virus. At high ambient temperatures, chickens appear to be more susceptible and to more frequently develop neurologic signs (Sinha, 1957). Some of the velogenic strains are capable of producing death following the introduction of only a few infective particles; other strains require a million or more infective units to induce death; some strains are nonfatal even in enormous doses. The route of infection modifies the nature of the pathogenic process. Natural routes of infection, such as intranasal, oral, and ocular, including aerosol forms, are most frequently associated with development of respiratory disease. Intramuscular, intravenous, and intracerebral administrations usually enhance the neurovirulence of the virus.

A sequence of events following introduc-

of incubation of naturally infected eggs. The chicken embryo is susceptible to the virus of ND and other diseases during its successive stages of development, yet the effects produced at each stage are markedly different due to differentials in specific enzyme and humoral activities (Blattner and Williamson, 1951; Buddingh, 1952). However, some of the lentogenic vaccine strains (B_1, LaSota, F) inoculated in 10-day embryonating eggs may require 90 to 150 hours to produce death, and by direct intracerebral inoculation may kill less than 10 per cent of susceptible day-old chicks (Hanson and Brandly, 1955).

An outbreak of ND may be so acute and severe as to kill all, or nearly all, of the birds in a flock within 3 or 4 days. At the other extreme, the disease may be so mild that symptoms are scarcely noticeable or they may be absent and the disease subclinical in nature. Natural and acquired differences in both the virus and the host, as well as known dosage and various environmental factors, appear largely to explain differences in the nature and course of the disease.

The incubation period of ND after natural exposure has been reported to vary from 2 to 15 days, or even longer, with an average of 5 to 6 days. The incubation time, as well as severity of the disease, decreases gradually from hatching to maturity. The response of offspring of immune hens may be altered favorably by the passive protection afforded by antibodies derived from the yolk. With heavy exposure to virulent NDV, rapidly developing prostration and early death may be observed to the exclusion of other signs.

Signs Among Baby Chicks and Growing Chickens

The most common signs of ND in chicks are respiratory in nature—gasping (Fig. 22.2), coughing, and hoarse chirping. Sometimes there is complete aphonia. Depression, partial or complete inappetence, and increased thirst in the early stages, and huddling are common expressions. Nervous

signs, including partial or complete paralysis of the extremities, muscular tremor, and rhythmic, clonic spasms, usually follow but sometimes accompany the respiratory signs. Peculiar attitudes, including torticollis (Fig. 22.3), opisthotonus, emprosthotonus, and lateral deviation of the head, are associated with a variety of abnormal movements, e.g., rearing, somersaulting, walking in circles, falling.

Partial or complete motor paralysis of one or both legs was the only sign observed in several outbreaks of ND among turkey flocks (Gray et al., 1954).

Whereas respiratory involvement is common, all chicks of an affected brood may not show this sign; a few individuals may develop nervous manifestations only, and occasionally neither sign may be entirely absent in a brood. The respiratory symptoms usually persist in the brood for 2 to 3 weeks. Temporarily delayed growth or permanent stunting of development is a variable consequence of ND. Recovery rarely occurs after prominent nervous signs develop. Other clinical signs, at times obviously modified by complicating factors, have been described in young and old birds of other avian species as well as chickens affected with ND. In younger birds the mortality may range from zero to 100 per cent according to reports from various parts of the world.

Signs in Laying Flocks

The disease usually appears suddenly and spreads quickly through fully susceptible flocks. Respiratory distress of varying severity, with coughing and gasping by a few or virtually all of the birds, is generally the first sign. In some flocks and outbreaks, respiratory involvement may be so mild as to be scarcely detectable, or it may be absent. These manifestations are identical with signs of bronchitis and closely resemble those of laryngotracheitis. Depression and impaired appetite accompany the respiratory difficulty. The flock may virtually cease eating within the week following onset. Concurrently, egg production may

icity occurs within periods of 5 minutes to 6 hours. At 37° C., hours or even days are required to induce these changes. At 20° C. and 8° C., months or years pass before all reactivity of the virus is lost.

NDV is destroyed by exposure to ultraviolet light rays in a similar fashion to other myxoviruses (Oppenheimer *et al.*, 1944). It has a rather broad stability in the presence of varying hydrogen ion concentrations. The infectivity is retained for many hours at a pH as low as 2 and as high as 10 (Moses *et al.*, 1947).

The inactivating effect of chemicals is very much dependent upon substances in the suspending medium, large quantities of protein reducing the effect of chemicals and delaying the inactivation of the virus. Formalin, beta-propiolactone (Mack and Chotisen, 1955), and phenol are useful in destroying the infectivity without severely damaging the immunogenicity of the preparation. At low temperatures, dilute formalin will destroy the infectivity without markedly affecting the hemagglutinin and without any measurable effect upon the immunogenicity of the preparation. Most known viricidal chemicals will destroy NDV (Tilley and Anderson, 1947; Beamer and Prier, 1950; Cunningham, 1948).

Destruction of the stability of NDV in nature is greatly dependent upon the medium in which the virus is present, e.g., decaying carcasses, feces, drying or fermenting matter, or mucous droplets in air (Zakomirdin, 1963; Walker *et al.*, 1953; Olesiuk, 1951; Mickalov and Vrtiak, 1963; Boyd and Hanson, 1958). The proteinacious matter may not only be protective but it may nullify the action of the disinfectant. Environmental conditions, particularly warm temperature and solar radiation, facilitate the destruction by chemicals. Freezing temperatures suspend most inactivating procedures. Disinfection should follow physical cleaning and, in winter, should be carried out only in the presence of supplemental heat.

A strain of NDV is a culture that has been recovered from a chicken or other host by inoculation of a suitable laboratory system such as embryonated eggs or tissue culture. Such a culture or isolate can be called a strain without signifying that it is necessarily distinct or different from any other culture or isolate (International Rules of Nomenclature). Almost all strains of NDV that have been characterized on the basis of their physical stability and their biological properties have been shown to differ slightly or markedly from one another (Hanson and Brandly, 1955). From such strains, one can isolate sublines which are differentiable from the parent culture on the basis of plaque morphology (Granoff, 1964; Schloer, 1964). These lines are usually differentiable also on the basis of their virulence for selected host systems and selected physical characteristics, and they may differ in their antigenicity. Sublines may also be separated by selective properties such as ability of a portion of a viral population to resist thermal shock (Goldman and Hanson, 1955). A subline may be separated from a culture by its ability to propagate in a given host system (Brueckner *et al.*, 1950; Romarov and Goldsmit, 1946). With few exceptions, strains are composed, on initial isolation, of heterogeneous populations that are separable on bases of plaque type and ability to surmount physical or biological hurdles. These population segments presumably arise through mutation (Granoff, 1964) and persist with varying degrees of success so that the population is the sum of the surviving mutants at any one time.

The heterogenicity of Newcastle strains suggests a built-in adaptability which has survival value to the virus in nature. It also suggests the desirability of formulating vaccines by selection of the most antigenically competent viral lines.

NATURE AND COURSE OF THE DISEASE

Proof is lacking that NDV, within an egg laid by an infected hen, may survive in and permit hatching of an infected embryo. Nevertheless, infection and death of embryos may occur during the first 4 or 5 days

FIG. 22.3 — Nervous symptoms of Newcastle disease.

from zero to 100 per cent among laying flocks have been reported from various parts of the world (Brandly, 1950).

Effect on Egg Quality and Production

The laying of soft or imperfectly shelled eggs early during attacks of ND in laying flocks may be followed after recovery by temporary and permanent abnormalities of egg quality. Lorenz and Newlon (1944), Berg et al. (1947), and later others reported on the basis of field findings and observations of trap-nested hens of absent or tremulous air cells, watery albumen, and rough, discolored, and chalky shells, and of abnormally shaped eggs. A high percentage of the hens laid abnormal eggs up to 45 days after an outbreak of ND (Lorenz and Newlon, 1944). The loss of albumen quality and shell abnormality tended to be permanent. Parnell (1950) found that eggs from hens that had experienced an attack of ND had low albumen indexes

when fresh and at successive periods up to 64 weeks in cold storage. Such eggs, as compared to those from hens not previously affected, did not keep as well in storage and the loss from inedible eggs was greater. Stuck yolks, which usually accompany watery albumen, accounted for most of the loss. The yolk quality was not altered. Parnell observed that during active ND infection some hens seem to lose their ability to secrete thick albumen while others do not. Knox (1950) reported on the effects of an epizootic of ND among crossbred and standardbred Rhode Island Reds. Comparisons of egg production and size over a 2- and 3-year period, including the outbreak, indicated the epizootic probably had a 12 weeks' effect on egg production before normal percentage production was attained for that time of year. Egg production decreased from an expected 60 per cent to 17 per cent for outbred Rhode Island Reds, to 40 per cent for incrossbreds

FIG. 22.2 — Respiratory symptoms of Newcastle disease.

drop precipitously to a low level or to zero Respiratory signs may recede or disappear entirely within 2 to 3 weeks, although apparent spread and persistence of ND may be prolonged, especially in flocks partially resistant as a result of prior infection or vaccination. Nervous involvement may appear as in chicks, although the proportion of cases is usually lower among the older stock. In the absence of complications, the appetite may return to normal after several weeks, and egg production has been reported to reach preinfection levels 1 to 8 weeks after an outbreak (Beach, 1917; Biswal and Morrill, 1954). If molt is induced, especially after production is well started in the pullet flock, return to production may be further delayed. In other instances, the egg yield as well as egg quality of the flock is permanently impaired.

In the more severe outbreaks, depression and "septicemialike" prostration are early prominent signs. The entire flock may sit huddled on the roosts and floor, eating little or nothing. A number of yard or floor eggs, mostly with soft or imperfect shells, are usually laid. A profuse fluid diarrhea often accompanies the early febrile reaction which, with rapid dehydration of the body, soon becomes sparse and almost exclusively urate in composition. Even in such outbreaks, the less severely affected birds may recuperate rapidly, marked improvement being apparent within 24 to 48 hours. The central nervous system is involved in a variable number of birds. Clonic spasms and partial or complete paralysis of one or several extremities, the head and neck, or the entire body, especially combinations of spasms and paralysis, are quite characteristic of ND. The consequence of these and other signs, e.g., torticollis and incoordination, may not be early death, but individuals so affected may be classed as "mortality" since recovery is rare. Mortality rates ranging

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and 34 per cent for crossbreds. The decrease in per cent of egg production for all groups during the epizootic was sufficient to lower the annual egg production, but to a lesser extent than was anticipated from the decrease observed during 12 weeks of low production. Average egg weights for the 3 months, as well as the annual egg weights of all groups, were decreased to a small but appreciable extent. A considerable time elapsed before birds were able to overcome the effects of ND on egg weight.

Upon infection with ND, some pullets laid imperfect and soft-shelled eggs (Biswal and Morrill, 1954). The eggs from certain pullets had a definite reduction in shell weight and thickness which persisted at least through the 56 days after infection.

Clegg and Mueller (1951) concluded that eggshell abnormalities resulting from ND infection were due to malfunction of the shell-producing uterus of the previously infected hen.

EPIDEMIOLOGY

Important in the ecology of ND virus and, hence, in the epidemiology of ND are the high resistance of the virus to adverse environment, the facility of its spread by various means, including air-borne routes or mechanisms, and its relatively broad and apparently expanding host spectrum (Beaudette, 1943, 1949; Brandly, 1950, 1953; Brandly et al., 1946a).

From the standpoint of the hosts' ecology, various states from complete refractivity to ready susceptibility to infection and, therewith, the occasional development of a balanced, though highly insecure, host-virus relationship are of major consequence. Environmental factors, especially husbandry and industry practices, obviously influence the ecology of the host, and these, together with other factors not already referred to in previous sections, may be considered briefly in the epidemiology of ND.

Season

Earlier reports indicate that ND prevailed mainly during the fall and winter seasons, tending to disappear largely during the warmest weather in enzootic areas (Beaudette, 1943; Brandly et al., 1946a). Davis et al. (1950) observed from a field survey that the reaction to living ND vaccine, mild and satisfactory in the summer of 1948, became so severe during the following winter that vaccination was discontinued as a practice in the area. "Breaks" after vaccination, regardless of the type of living or inactivated vaccine used, were greater during the winter season. Sinha et al. (1957) studied the severity of ND in 6- to 7-week-old chickens acclimatized to different environmental temperatures. After infection with viral aerosols, the mortality at 85°–90° F. was 100 per cent; at 70°–75° F., 95 per cent; at 50°–55° F., 75 per cent, and at 32°–35° F., 55 per cent. The incubation period was shortest at the warmer temperatures. Nervous signs of ND predominated at warmer temperatures; the respiratory signs were more pronounced at the colder temperatures.

While climatic factors may mediate susceptibility to ND infection, it would appear that husbandry practices which provide successive broods of susceptible chicks on contaminated premises throughout the year inordinately favor perpetuation of the infection.

On the basis of various evidences of prior unrecognized, mild, or subclinical ND in the United States, Beaudette (1952) believed that the obvious clinical disease may have been provoked by cold, adverse weather.

Age

More recent reports substantiate earlier observations which indicated that birds of all ages and breeds are susceptible although substantially less so with advancement to maturity (Beaudette, 1943; Brandly, 1953; Brandly et al., 1946a; Crawley, 1954). Earlier compilations of well-

documented evidence (e.g., Brandly, 1953) emphasize the much greater hazards of severe vaccination-induced ND among chicks one to several days of age than among older birds. Congenital immunity as a modifying factor of susceptibility and epidemic behavior has already been alluded to.

Host Range

Recent evidence of ND infection in several previously unreported species indicates that the host range or spectrum of ND is expanding, perhaps in part the result of adaptative mutation. Aside from the wider distribution of the infection and increased opportunity for exposure which favor its extension to new hosts, there is a greater awareness of the prevalence and adaptability of ND virus. It seems logical that the paucity of recorded observations of mild and subclinical ND in chickens and other susceptible as well as more refractory species may be largely accounted for by superficial and incomplete observations and the inadequacy of earlier diagnostic techniques.

A number of additions can be made to the species reported earlier as affected during natural outbreaks, namely, chickens, turkeys, guinea fowls, ducks, geese, parrots, pigeons, pheasants, partridges, crows, sparrows, mayas, and martins as well as unidentified species of free-flying birds (Beaudette, 1948, 1950; Brandly et al., 1946a). Nevertheless, the evidence of susceptibility of several wild species was not isolated. Initially reported isolations of ND virus from other species include: a natural case of the disease in a nestling European starling (Sturnus vulgaris) in New York (Gillespie et al., 1950); recovery from the bone marrow of a gannet (Sula bassana) in the Orkneys (Wilson, 1950); from a great horned owl (Bubo virginianus virginianus) in Ohio which showed nervous involvement (Ingalls et al., 1951); from an osprey (Pandion haliaetus) and three parakeets

(Palcornia) in Holland (Zuijdam, 1952a); and from the jackdaw (Corvus monedula) (Keymer, 1961). Naturally infected in an outbreak in a zoological garden were the little owl (Athene noctua), raven (Bucorvus sp.), white-tailed eagle (Haliaetus albicilla), and giant kingfisher (Dacelo gigas) (Schoop et al., 1955). Vrtiak (1958) reported recovery of the virus from a swan by Shah and Johnson (1959), from a nestling koel (cuckoo), and from a canary by Monda et al. (1961).

Avian species reported susceptible to inoculation infection are quail, sparrows (Beach, 1942; Pomeroy and Fenstermacher, 1948), grouse (Pomeroy and Fenstermacher, 1948), and the laughing dove (Kaschula, 1950).

Possibly indicative of the predictable behavior in species considered to be relatively resistant is the infection in ducks and geese. Earlier reports record both death and incomplete susceptibility of these species during outbreaks in fowl on the same premises and areas. Asplin's findings (1947), while supporting the substantial refractivity of ducks and geese to contact ND infection, revealed only temporary subclinical infection. Macpherson (1956b) infected a fledgling cormorant by feeding tissues of a hen dead of Hertfordshire strain ND infection. It remained well and its blood showed a high HI titer 7 days later; at 5 weeks following feeding, the titer was waning and became negative at 6 weeks. This author stated, "The short duration of immunity in the cormorants under experiment, as measured by the antibody content of their serum, indicates the possibility of repeated infection in this species analogous to repeated influenza in man. The low morbidity in comparison to the high morbidity in cormorant species may indicate a long established biological adaptation of ND virus to these birds and that the domestic fowl may, in fact, be only the secondary host."

Further experimentation tends to confirm earlier observations that few, if any,

species of birds are refractory to massive exposure with various strains by all routes. However, Placidi and Santucci (1956) state that carnivorous birds are apparently entirely resistant.

Determination of susceptibility to infection by feeding or aerosol exposure should afford a better indicator of liability to natural infection than parenteral inoculation would. Both pigeons and doves were found susceptible to severe and frequently fatal infection with strain GB (Boney, 1951) administered as an aerosol (Hanson and Sinha, 1952). Moses (1951) infected cowbirds, grackles, starlings, and English sparrows by aerosols of strain 11914. Sparrows were also subjected to aerosols of strain GB and contracted infection.

Recognition of the infectivity of NDV for man by direct and indirect contact and for various other mammals, naturally and artificially, is a recent significant development. Since Burnet in 1942 recorded the first human case, which resulted from accidental introduction of infected allantoic fluids into the conjunctival sac, a number of human cases have been recorded. To those summarized by Keeney and Hunter (1950), and among which 11 were verified by virus isolation, have been added many reports including those of the Minnesota group (Pomeroy, 1951; Quinn et al., 1952; and Lippmann, 1952), and the summary report of more than 100 cases throughout the world recorded in 53 papers during the period 1943 to 1955 (Hanson and Brandly, 1958). The Minnesota outbreak involved 40 of a total of 90 persons employed in evisceration of poultry at a slaughtering plant. Virus was recovered from 4 of 10 individuals examined while showing acute symptoms. Quinn et al. (1952) record 2 cases with ocular signs and a third with only general symptoms. The latter yielded virus from the nasal secretions and one of the others from the urine as well as the blood, nasal secretions, and saliva. Generalized infection without ocular involvement and recovery of the virus from the saliva had been recorded previously by Mitchell and Walker (1951) while the presence of the virus in the urine of man had not. Recognition of ND as an occupational hazard, especially to poultry eviscerators and to persons preparing ND vaccines and administering the living vaccines in the form of sprays and dusts (Dardiri et al., 1962), and of its potential human-to-human spread should serve as a major impetus for the poultry industry and the veterinary medical profession to suppress the malady at its source and eventually to eradicate it. Conjunctival reinfection of man within 4 weeks (Freymann and Bang, 1949) and after 4½ years (Jacotot et al., 1955) has been recorded.

A number of species of mammals including mice, hamsters, monkeys, dogs, guinea pigs, rabbits, ferrets, swine, calves, cats, bats, shrews, hedgehogs, and chinchillas have developed clinical manifestations after artificial exposure to NDV chiefly by parenteral routes. In several species, e.g., swine and sheep (Hofstad, 1950; Brueckner et al., 1950) and monkeys (Meyer and Mack, 1946; Wenner and Lash, 1949; Reagan et al., 1947), morbidity or death, or both, resulted only after intracerebral inoculation, not by intravenous and other routes. Six strains of NDV identified only according to the state of origin, were infective for cave bats (Myotis lucifugus) by intranasal instillation as well as by intracerebral, intraperitoneal, and intradermal injection (Reagan and Brueckner, 1951).

The reported capacity of certain strains of NDV to infect various mammals including man, experimentally, or in nature, i.e., cats (Bolin, 1948; Giltner, 1950; Report of Director, Purdue Agr. Exper. Sta., 1950), and man further stresses the real and potential range of infectivity of the virus.

Modes of Spread

Various reports and surveys (Beaudette, 1943, 1950, 1951; Brandly et al., 1946a; Kaplan, 1949; Blood, 1950; Brandly, 1950, 1953; and others) indicate that natural spread of ND has been chiefly through the media of exudates, excreta, and offal of infected birds. The digestive and respiratory routes obviously constitute the major chan-

nels of natural infection, although entry of NDV by the ocular and cloacal routes may be quite common.

Traffic in live birds, including inapparent cases and recovered birds as well as clinical cases, has often accounted for dissemination of infection, frequently over long distances (Beaudette, 1943). Initiation of the 1947 epizootic of ND in England (Gordon and Asplin, 1947) like the 1942 outbreaks in Düsseldorf and Arnsberg, Germany (Brandly et al., 1946a) was ascribed to the importation of market poultry. In the English outbreak, uneviscerated infected carcasses from Hungary were incriminated. In 33 per cent of 540 outbreaks there was a history of access to swill or butcher's waste, the former being found in some cases to contain the offal of imported carcasses. Traffic in live poultry accounted for 42 per cent of the first outbreaks. During later outbreaks, Gordon et al. (1948) observed that less than 5 per cent of the outbreaks arose from contaminated swill, while those originating from traffic in live birds increased to over 70 per cent. Dobson and Simmins (1951) demonstrated NDV in 50 of 135 samples of skin from eviscerated, imported poultry carcasses including chickens, turkeys, ducks, and geese. The fact that the isolants appeared to have been of a highly pathogenic type and that infectivity of survivors of severe ND is usually of short duration suggested to Asplin (1952) that these findings could be explained by surface contamination of the skin or by subclinical infection of partially resistant fowls with the virulent virus in the packing stations of the country of origin. Koser (1942) believed that certain flock outbreaks resulted from contamination introduced by irrigation of meadows.

Introduction of ND into the Hawaiian Islands and South America was attributed to the importation of infected chicks (Adler et al., 1951; Divo, 1950). Importations of ND have been ascribed to game or other birds (Report of Chief, B.A.I. 1950; Jansen et al., 1949; Zuijdam, 1949).

The previous evidence of air-borne spread of ND was substantiated by the work of DeLay et al. (1948) who recovered the virus from the air of a poultry house harboring an infected flock. Artificially created aerosols of the virus have been shown by Hanson and Sinha (1952), Sinha et al. (1952, 1954), and others to serve as a ready means of transmitting the virus.

More recent reports affirm the respiratory tract (Kohn, 1955, 1959) to be the most important and susceptible to ND infection yet an ND epidemic in Nigeria has been described that did not spread from pen to pen via the air, nor rapidly in the feed, but readily via infected drinking water (Kaschula, 1961b).

The presence of NDV in eggs laid during the preclinical and early acute stages of the disease (Van Roekel, 1946; Jungherr, 1946; Prier et al., 1950) may permit, through breakage, the contamination of crates, incubators, and other objects as well as the surrounding atmosphere. The virus, which has been found in about a third of the eggs laid during the acute phase of a flock outbreak or after live virus vaccination (Beaudette, 1948), would appear to prevent development or kill the embryo of fertile eggs (Doll et al., 1950a; Hofstad, 1949b). The report of DeLay et al. (1947), suggesting parent-to-offspring transmission as a result of finding virus in 4-day-old chicks from eggs laid during an outbreak, is interesting but requires confirmation. Living ND vaccines, especially those of relatively high pathogenicity, may serve to introduce infection into or upon premises from which it may spread directly via vaccine-infected birds or indirectly by contamination of air, water, and various objects. Fowl pox, laryngotracheitis, or other vaccines contaminated with ND virus (Zargar and Pomeroy, 1950; Hanson, 1953) have spread ND.

Carriers and Vectors

While the fowl in the early incubative and frank stages of ND is recognized as the most potent source of infection, it has become evident that the convalescent or recovered individual may sometimes harbor

and eliminate the virus for substantial periods. Several investigators have reported detection of NDV in recovered chickens or in eggs laid during periods of 2 to 4 months following exposure or clinical recovery (Beach, 1912; Jungherr and Terrell, 1946; Schoening and Osteen, 1948; Walker and McKercher, 1954). Pomeroy (1948) reported NDV recovery from a chicken showing illness 17 months after having undergone recognizable ND infection. Beaudette (1948) reported isolation of NDV from a flock at 5 weeks of age and again when it was in 10 per cent production. The possibility of reinfection in both these instances was, however, not excluded. Walker and Powell (1950) reported that normal hens, placed in contact with a rooster infected 6 months previously, developed infection after 5 weeks' exposure. Brandly (1945) observed that susceptible hens, placed with an immune cockerel that had been inoculated with virus several days previously apparently contracted the disease from the cockerel. Prier et al. (1950) failed to recover NDV from eggs laid by immune hens 1 to 26 days after heavy challenge. However, Zuijdam (1952b) found that flocks of chickens partly immune, as a result of vaccination with a killed or living vaccine, excreted virus for 33 and 19 days after a subsequent challenge with virulent virus. Virus was not shed after vaccination with two other living vaccines and subsequent challenge. Asplin (1952) found that fowls vaccinated with strain F, of low virulence, failed to show signs on challenge but they excreted virus. Dinter and Bakos (1953) and Woernle and Brunner (1957) reported similar results, the former of transfer of NDV to pen contact birds. Schmidt and Bindrich (1956) observed virus excretion in clinically immune fowl after Hertfordshire virus vaccination only when the HI serum titer fell below 1:16 or 1:20 and the SN titer was lower than the HA.

Numerous other observations indicating failure of infection to occur, even after frequent or prolonged contact of susceptible and recovered birds, suggest a low rate of recovered carriers. Nevertheless, with the development of predominantly pneumotropic strains of virus and the occurrence of largely nonfatal infections or of natural reinfections, the possibility of an increase in carrier rate may be anticipated.

Gordon et al. (1948) concluded that the introduction of healthy turkeys from affected areas apparently accounted for the origin of some ND outbreaks. Other birds, especially free-flying species (sparrows; turtle, striped, and laughing doves; and cormorants) appear to have been incriminated as vectors or carriers by the observations of Kraneveld and Mansjoer (1950), Mansjoer (1961), Kaschula (1950), Gustafson and Moses (1953), Hartwigk and Nitschke (1957), Hanson and Sinha (1952), and Macpherson (1956b).

The presence of virus in the feces of various mammals, e.g., mice, rats, dogs, foxes, or cats for periods up to 8 days after they had eaten NDV-infected embryos or fowl carcasses has been demonstrated (Polci and Silvagni, 1954; Zuijdam, 1951; Walker et al., 1954; Baczynski, 1959; and others).

Arthropods have not been incriminated as vectors of ND. Komarov (1940) found ticks (Argas persicus) abundant in an infected flock, but allowing them to feed on a healthy fowl did not transmit the disease. By inoculation of susceptible fowl with ticks from a diseased bird, the virus was found to survive in or on the tick for 7 but not 10 or 13 days. Bolin (1948) isolated NDV from common chicken lice collected from hens 33 days after subcutaneous injection with the virus. Hofstad (1919a) obtained negative results by transfer of mites (Liponyssus sylviarum) from infected chickens, but inoculation with washed mites that had fed on infected birds caused infection.

Mansjoer (1961) found that the virus remained viable in the Indonesian giant snail for several weeks and, hence, could be a source of infection.

DIAGNOSIS

A combination of means or methods is usually required for definitive diagnosis of Newcastle disease. These may embrace clinical examinations, gross and histological observations, isolations of the virus, virus-serum neutralization tests, hemagglutination, hemagglutination inhibition, hemadsorption, and specific immunity tests (Beaudette, 1943, 1951; Brandly et al., 1946b; Jungherr et al., 1946; Cunningham, 1952; Bankowski et al., 1959; and others).

Clinical Examination

The signs of ND described under "Nature and Course of the Disease" obviously closely resemble or are identical with those of a number of infectious diseases. Bronchitis, laryngotracheitis, coryza, leukosis, mycosis, pullorum disease, and the so-called chronic respiratory disease (Delaplane and Stewart, 1943) and infectious sinusitis of turkeys cause gasping and other respiratory signs indistinguishable from those frequently seen in birds and flocks affected with ND. The common nervous expressions of ND—lameness, paralysis, incoordination, torticollis, etc.—are often similar to or identical with those seen in the following diseases; neurolymphomatosis (fowl paralysis), avian encephalomyelitis (epidemic tremor), riboflavin deficiency (curled toe paralysis), vitamin E deficiency (encephalomalacia or crazy chick disease), and sometimes vitamin D (rickets) and vitamin A deficiencies; also botulism, heavy metal poisoning, and, according to Placidi (1954), corn cockle (Lychnis githago) poisoning.

Distinction between ND and respiratory diseases such as bronchitis, laryngotracheitis, and chronic respiratory disease (mycoplasmosis) is particularly difficult in flocks of growing and laying birds where the rather typical nervous manifestations of ND are lacking. Nervous signs may be absent or fail to appear until late in the course of the outbreak, thus complicating or preventing a clinical diagnosis. A pre-cipitous drop or cessation of production among layers and the development of nervous signs following respiratory involvement, especially in chicks, often suggest ND.

Gross Lesions

The pathologic changes of ND, chiefly hemorrhagic and inflammatory in nature, vary greatly in location, severity, and extent from bird to bird and among flocks and outbreaks (Beaudette, 1943; Jungherr et al., 1946). The variability in tropisms and pathogenicity among and within strains of NDV, as already mentioned, has been the cause of much confusion and delay in diagnosis and in the search for a satisfactory name for the disease. Likewise, variation in the response of the host animal associated with such factors as age and partial immunity often contributes materially to differences in lesions. Peracute or acute cases may show predominantly hemorrhagic pictures with severe and extensive involvement of the proventricular submucosa and the lymphoid patches and follicles of the intestine and, to a lesser extent, the gizzard (Doyle, 1927; Beaudette, 1943; Jungherr et al., 1946; Kaschula, 1961b). Hemorrhagic necrotic involvement adjacent to the lymphoid plaques of the intestine, including the so-called cecal tonsils, is a significant feature of infection by the more pathogenic strains with endotheliotropic properties (Jungherr et al., 1946).

Focal hemorrhages of the serous membranes, while occasionally described, are not as prominent or constant as in fowl plague and pasteurellosis. The markedly hemorrhagic conjunctivitis sometimes seen in man (Shimkin, 1946), the occasional congestion and hemorrhage of the conjunctiva in fowl (Bankowski, 1946; Crook, 1951; Santoni and Bonaduce, 1932), as well as the hemorrhagic encephalitis occasionally encountered in inoculated chicken embryos (Burnet, 1942; Jungherr et al., 1946) are further expressions of the endothelial

affinity of NDV. In acute forms of ND, the spleen may be mottled or quite pale and shrunken, the latter an apparent consequence of hemorrhage into the body tissue.

Cloudiness of the air sacs, with a film of grayish or yellowish exudate, is a frequent but not constant lesion of infection with distinctly pneumotropic strains (Beach, 1912) and has been commonly seen with various strains proposed for use as ND living virus vaccines. The serositis of the pulmonary air sacs has a marked tendency to progress to the pericardium and visceral epicardium with consequent pericarditis and resultant marked thickening, opacity, and caseous exudates. The subacutely or chronically affected air sacs may be thickened and show fibrotic "scarring."

Congestion and mucous exudation, generally quite mild, are often present in the tracheas of birds which have shown respiratory signs, but these changes do not serve for differentiation of ND from other respiratory infections

Biswal and Morrill (1954) found that ND infection of laying pullets may result in oophoritis, with marked degeneration or atresia, and lymphocytic hyperplasia. In the pullets studied, there followed arrest of ovulation and subsequent oviposition.

Clouding or graying of the eyes, as described in a few cases, may result from suspension of inflammatory cells in the fluid of the anterior chamber. In rare cases, vesicles form on the wattles and comb and these may indicate a dermotropic character of the ND virus (Brandly, 1945; and others)

Newcastle disease infection of the embryonating chicken egg, by chorioallantoic membrane inoculation, first described by Burnet and Ferry (1934), frequently results in the development of minute, fine, gray foci in the exposed portion of the membrane or of plaquelike gray thickenings with satellite foci. Congestion of the feet and skin varies from slight to moderate. Distinct petechial, or slightly larger, hemorrhages of the skin of the embryo occur quite commonly with some strains, less often with others. Infrequently, the embryos may show marked cranial hemorrhage or "hemorrhagic encephalitis." Gross congestive and hemorrhagic involvement of the yolk sac is present in a considerable proportion of specimens. This change has not been seen in fowl plague, pox, bronchitis, and laryngotracheitis infections of the chicken embryo (Jungherr et al., 1946).

Microscopic Lesions

Lesions considered to be characteristic of infection with NDV vary with the organ, virus strain, and other factors involved. They are essentially necrotizing in nature in the spleen, liver, gallbladder, intestine, and heart, and of a proliferative character in the lung, iris, and central nervous system. The serous membranes of the thoracic and abdominal cavities manifest secondary changes of inflammation and cellular infiltration.

Characteristic gross hemorrhagic, necrotic foci of the lymph follicles, patches, and cecal tonsils were found to occur near or in the normal lymphoid aggregates. Recent foci have been observed also in the interfollicular tissue of intact lymphoid follicles. Well-developed foci are usually situated in the subepithelial region of the mucosa and frequently bulge into the lumen, virtually occluding it. Complete cell necrosis and conspicuous hemorrhage are found at the center of the focus while the border shows capillary congestion and extravasation. The foci tend to produce a partial sloughing of the affected mucosa overlaid by serous exudate containing bacterial clumps; the foci usually extend to, but rarely beyond, the muscularis mucosa. Most of the intestinal wall is involved, but the serosa is left intact, although the adjacent mesentery sometimes shows capillary congestion and extravasation. The tissues of and adjacent to the lymphoid aggregates of the heart, liver, gallbladder, and proventriculus occasionally also show necrotizing and hemorrhagic lesions.

The lung lesions are primarily proliferative and secondarily exudative in chat-

acter. Hyperplasia of the alveolar wall cells, i.e., the epithelial covering, the endothelial lining of the capillaries, and the connective tissue septa, is indicated by the relatively numerous mitotic figures present. The proliferative process may partially or completely obliterate the alveolar space with consequent consolidation and interstitial pneumonia. The exudative coronary changes consist of cellular and serous accumulations in the peribronchial alveoli with extension into the regional tertiary bronchus. The pulmonary and abdominal air sacs reveal secondary inflammatory changes characterized by edema and mononuclear and some heterophil cell infiltration accompanied by metastatic thickening of the mesothelium. Fibroblastic proliferation is a common sequel of chronic infection. The iris also occasionally shows cellular infiltration. An interstitial pancreatitis has been observed with a few viral strains (Jungherr et al., 1946).

Both the ovary and various sections of the oviduct of laying pullets may show inflammatory reaction to NDV. Oophoritis was characterized by marked degeneration and subsequent lymphocytic hyperplasia. In the earlier stages, heterophilic infiltration, edema, and capillary hemorrhage were prominent; later there was lymphocytic hyperplasia. However, only the shell-secreting portion appeared to suffer functionally upon return to production (Biswal, 1954; Biswal and Morrill, 1954).

Among birds which survive challenge inoculation following vaccination, a considerable proportion may show gross residual lesions such as scattered gray foci of the spleen and liver and, particularly, focal fibrosis of the air sacs. Histologically, there are discrete areas of consolidation of the lung often associated with fibrosis; exudative lesions are more common than in normal birds. There seems to be an increase of noncircumscribed lymphocytic aggregates, particularly of the liver and kidneys. Evidence of stress on the renal function during immunization may be seen in the considerable areas of tubular de-

generation and regeneration (Jungherr et al., 1946).

The central nervous system lesions of ND have been described by various authors. Only thickening and proliferation of the vascular endothelium accompanied by early neuronal changes are usually seen in recent lesions, while characteristic glial clumps are more common in the older ones. Loci of predilection are the lumbar cord, medulla, and cerebellar nuclei. The glial foci of the cerebellar molecular layer may be indistinguishable from those occurring in avian encephalomyelitis, although they occur unassociated with marked perivascular cuffing and axonal reaction of the neurons characteristic of the latter disease.

Virus Isolation

As with other infectious diseases, the unequivocal method of diagnosing ND is the isolation and identification of the causative agent. Serologic and pathologic examinations may suffice to confirm suggestive histories and signs in areas where ND is enzootic, but new foci or extensions of the disease usually require recovery and identification of the virus. Likewise, ND infection of avian and mammalian hosts, including man, may induce typical or previously unidentified signs and symptoms. Signs or serologic evidence may be lacking entirely (Quinn et al., 1952), or the serologic findings, as either mumps in man where the serum may neutralize ND virus, may be misleading (Kilham et al., 1949; Evans, 1955; Bang and Foard, 1956).

Specimens for attempting isolation of the virus should be selected from cases in the incubative and early clinical stages of the disease (Brandly et al., 1946b). Younger individuals are more likely to yield virus (Brandly et al., 1946b; Beaudette et al., 1948, 1949a), and one should utilize suitable specimens of tissues most likely to have a high viral content, e.g. spleen, lungs, respiratory exudate. The more pathogenic and invasive strains are more widely distributed and present in greater

concentration in the various tissues and organs (Hofstad, 1951; Karzon and Bang, 1951; Sinha et al., 1952; Baskaya et al., 1952; Report of the Poultry Disease Subcommittee, 1963). The rapid disappearance from, or masking of the virus in the tissues of the host usually follows or coincides with the development of demonstrable circulating antibodies and detectable immunity to ND (Brandly et al., 1946b; Hofstad, 1951; Report of the Poultry Disease Subcommittee, 1963). Yet the virus may be isolated from the brain and excreta when circulating antibodies are present (Walker and McKercher, 1954).

Interference phenomena, e.g., of NDV by bronchitis virus after simultaneous exposure, may lead to erroneous results (Hanson et al., 1956). The recovery of NDV from the urine of an occupationally acquired human case emphasizes its wide distribution in the body and excretions and the necessity for adequate sampling in the search for it (Quinn et al., 1952).

The embryonating chicken egg is, for obvious reasons, preferred to the chicken or other animal for isolation of NDV. Details of the procedures of isolation, as described by various workers, point out the essential steps, precautions, and pitfalls (Bureau of Animal Industry, 1916a, 1916b; Brandly et al., 1946b; Beach, 1948; Beaudette et al., 1948; Thompson and Osteen, 1948; Report of the Poultry Disease Subcommittee, 1963).

As a rule, fertile eggs, preferably from healthy nonimmune hens, which have been incubated for 9 to 11 days at 37°–38° C., are injected in the allantoic chamber with suspensions of suspected tissues, free of bacteria. Antibiotic agents, usually either penicillin or streptomycin or more commonly a mixture of the two, are added to the inoculum to suppress or destroy bacteria which may be present (Brandly et al., 1946b; Thompson and Osteen, 1948; Beaudette et al., 1948). The injected and suitable control eggs are returned to the incubator, candled once or twice daily for the subsequent 5-day period or until death

of all or a large part of the embryos. Embryonic deaths during the first day are considered to be due to trauma or nonspecific causes. Examination of embryos dying subsequently may reveal some or all of the changes described under "Lesions," although congestive or hemorrhagic changes may be poorly defined or absent during initial or early egg passages of NDV. Clear, bacteria-free, allantoic fluids may then be tested for agglutinative activity for chicken erythrocytes and, if positive, it should be determined whether the phenomenon is inhibited by known ND immune serum. Identification of NDV may be accomplished, also, or confirmed by means of specific virus neutralization trials of the suspected egg fluids, or more directly, of the tissue suspensions, by employing mixtures of the ND immune serum and the unknown tissue for inoculating the embryonating egg as the test organism.

The use of susceptible chickens for isolation and identification of NDV may be expedient and desirable under certain circumstances where adequate isolation can be maintained. Much larger inocula may be employed than with eggs and possible difficulty in adaptation of the virus to the latter avoided (Beach, 1942; Brandly et al., 1946b; Geurden et al., 1950; Report of the Poultry Disease Subcommittee, 1963).

Interest in and recent progress with culture of NDV and other viruses in different types of tissue cultures of various cells, e.g., chicken embryo and adult cells, monkey kidney, Ehrlich ascites tumor, HeLa, and others suggest their further use for diagnostic isolation and differentiation (Topacio, 1951; Bankowski and Boynton, 1948; Pereira and Gompels, 1954; Seiffert, 1955; Chanock, 1955; Scott et al., 1953; Flanagan et al., 1955; Levine and Sagik, 1956; Bankowski, 1957; Bankowski et al., 1959; Goldwasser and Kohn, 1957; Rubin et al., 1957; and others). Epithelial cells of chickens, whether or not in tissue culture, may show normal as well as abnormal microvilli, the latter after infection, and from which virus is eliminated (Bang, 1952). Particles small-

stable, nonspecific inhibitors (Karzon, 1956) must be recognized and guarded against. Aliquots of suspected tissues, triturated, if necessary, and processed for inoculation, are admixed with suitable quantities of antibiotic preparations, of ND immune serum and of normal serum, respectively. The suspected tissues alone may be inoculated also in an effort to detect bacterial or other pathogens, or contamination which was suppressed by the antibiotic substances.

Hemagglutination (HA) and Hemagglutination-Inhibition (HI) Tests

The finding by Burnet (1942) and Lush (1943) that NDV, like influenza, agglutinated the red blood cells of chicks and that such hemagglutination was specifically inhibited or neutralized by ND immune serum provided a simple and useful diagnostic method. The test for HA may serve as a rapid method for detecting NDV in the fluids or extracts of infected tissues of fowl as well as in the fluids of infected eggs (McClurkin et al., 1954). Monti (1952). Mitscherlich and Gürtürk (1952), Clark et al. (1955, 1957), Gardner et al. (1954), and Geurden and Devos (1955) demonstrated HI activity in the sera of ND infected chickens by adding NDV-treated chicken erythrocytes as a means of early and rapid diagnosis. The latter state that the serum-sensitized erythrocytes may be lyophilized and stored indefinitely for use. Hanson et al. (1947) showed that minimal NDV titers, at least of 10^6, are required if the infected allantoic or amniotic fluids are to induce hemagglutination. Bronchitis and laryngotracheitis viruses, while infective for the chicken embryo, do not cause hemagglutination, thus providing a means of differentiation from the HA-positive NDV (Brandly et al., 1946b).

As with the test for neutralization of NDV infectivity by specific immune serum, the test for inhibition or neutralization of the hemagglutinative activity of the virus (HI) gives quantitative as well as qualitative information. In the alpha procedure,

the virus suspension in serial twofold dilutions beginning with 1:5 is mixed with equal volumes of the serum under test and a 0.5 per cent suspension of chicken red cells. In the beta procedure, the serum is serially diluted and admixed with the red cell suspension and a constant quantity, e.g., 10 HA units of the virus (Brandly et al., 1947). The test, with adequate control tubes, is incubated at room temperature for 30 minutes and the results read on the basis of the degree or titer of HI. Typical, complete HA $(+ + + +)$ is represented by a continuous layer of cells covering the entire rounded bottom of the tube; a thicker layer, irregular in outline, covering a portion of the bottom is a partial or $+ + +$ reaction; a small central disk with a narrow granular fringe of agglutinated cells is a $+ +$ reaction; and a larger button, or disc, of cells with a narrow ring of agglutinated cells is classed as a $+$ reaction. Complete inhibition of the HA reaction, like the normal unimpaired settling of cells as in the control tubes, leaves a central compact button with regular edges. The highest dilution of virus giving a $+ +$ or higher reaction represents the titer of the virus, and the quantity of virus involved is considered a unit. Elution of virus from the cells and their subsequent release from the agglutinated layer causes a cascading or sliding of the cells to the bottom of tube and the formation of a disc, or button, as occurs when erythrocytes sediment normally. Rapid serum and whole blood HI tests have been devised and used with satisfactory results (Luginbuhl and Jungherr, 1949; Zargar and Pomeroy, 1949; Walker, 1952; and others).

The variables, both quantitative and qualitative, inherent to the HI test prompted efforts toward standardization (Report of Poultry Disease Subcommittee, 1963).

The HI test, although somewhat simpler and less protracted than the VN test, is usually considered less reliable than the latter. Neither the antibody nor the mechanism of reaction is the same in the HI and VN test as indicated by the sub-

stantially longer persistence of the VN principle or antibody and results of serum fractionation studies (Brandly et al., 1947; Hanson et al., 1950). An HI titer value of 10, according to the beta procedure, has been considered as of questionable diagnostic significance, one of 20 or higher definitely indicating prior infection of the bird. On the basis of their studies, Doll et al. (1950b) concluded that the HI response may differ according to strain of virus in time of development, titer, persistence, and with respect to route of infection and other factors. After several routes of infection, the HI titer rose to 10 or 20 in 5 to 6 days, indicating that complete inhibition at these titers was of diagnostic value. More than 90 per cent of the experimentally exposed thickens showed positive titers at the tenth day and were positive on the twelfth day. Direct and indirect complement-fixation tests have been utilized and found highly specific for serologic diagnosis of ND (Boulanger and Rice, 1953; Nitzschke, 1954; Brumfield and Pomeroy, 1957). Since specific anti-ND substances are demonstrable only after a period of 7 to 10 days following NDV infection, the use of serologic tests for earliest definitive diagnosis should be supplemented with appropriate HA tests using tissues of suspected birds, or fluids of embryonizing eggs or tissue cultures inoculated with them (Valado, 1955; and others).

Immunity Tests of Living Birds

Definitive diagnosis of prior ND infection may be accomplished by inoculating with fully virulent NDV, suitable specimens from flocks suspected of having been infected at least a week previously. Susceptible controls are simultaneously inoculated with the previously titrated challenge virus. Serum samples should be tested prior to inoculation by SN or HI tests.

Other Tests

Superiority from the standpoint of sensitivity and economy of time and materials has been claimed for isolation or hemadsorption or both of NDV in HeLa, monkey

kidney, or chicken embryo (Bankowski et al., 1959; Matewa, 1960; André and Audebau, 1960; Jakubik, 1962) or other cells in tissue culture according to the procedure of Vogel and Shelikov (Shelikov et al., 1958). The fluorescent antibody procedure allowed more rapid detection of NDV infection than did HI or regular egg inoculation according to Maestrone and Coffin (1961). Raggi (1960) reported a rapid plate agglutination test for ND.

PREVENTION AND CONTROL

The simplest and most logical measure against Newcastle disease and other infections is to prevent contact of the virus with the susceptible bird or mammal. A second and indirect method, applicable against certain diseases, is vaccination. The latter gives the animal a greater or lesser degree of protection against infection in case of exposure. The third and least satisfactory and least economical procedure is to attempt treatment of the animal after it is exposed or has become affected.

A combination of sanitary management, to reduce or prevent exposure, and of vaccination is often required to combat highly contagious diseases, such as ND, that have become widely established in a community, region, or country. However, both measures must be systematically carried out on an area, regional, state, or larger basis if control is to be reasonably and permanently effective. A minimum of 70 per cent of the flocks in an area must be included at the outset in the rigid sanitary program, combined with a satisfactory twice-a-year, or oftener, vaccination program.

Restriction of exposure to and spread of ND generally increases in proportion to expansion of the size of the flock, the rearing and maintaining birds of different ages on the premises, and the proximity of the housing units.

General compliance with a rigid sanitary program prevents ND where the poultry population is relatively small and flocks are some distance apart. Vaccination may be required as a supporting measure to sanita-

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General compliance with a rigid sanitary program prevents ND where the poultry population is relatively small and flocks are some distance apart. Vaccination may be required as a supporting measure to sanita-

tion in less concentrated production areas, and if so, it should be employed as a means toward eradication of ND.

Such a goal was projected (Report of the Subcomm. on Plans for Eradication of Newcastle Disease, 1959) but not implemented in the United States where the disease is endemic yet infrequently clinically acute or peracute. A regional program to eliminate NDV has been implemented in Maine (Chute and O'Meara, 1963). Continuous application of the slaughter policy against ND in Great Britain in the face of reintroduction of infection and the prevalence of mild or subclinical infection has fallen short of success while imposing serious financial burdens on breeding and research stock perpetuation (Report of the Committee on Fowl Pest Policy, 1962). Vaccination with inactivated vaccines or with living lentogenic strains of low or limited diffusibility must be considered essential components of eradication programs especially where ND is widespread in an area or country (Osteen et al., 1961; Levine, 1962).

If it is not used properly and wisely, vaccination will engender among poultrymen an entirely disproportionate or false sense of security while constituting an appreciable year-after-year expense.

Natural selection toward development of greater genetic resistance is a consequence when ND becomes enzootic in a poultry population (Knox, 1950; Francis and Kish, 1955; Placidi and Santucci, 1956; Takamatsu et al., 1956; Cole and Hutt, 1961; and others). The practicality of intentional exposure to ND as the basis of a breeding program toward controlling ND has not been demonstrated. Environmental factors, especially higher temperatures, may greatly alter the severity of ND among a given stock (Francis and Kish, 1955; Sinha et al., 1957; and others).

The statistically significant differences in resistance to ND among six families as well as two strains of White Leghorn chickens which support early observations of such variability have been reported by Cole and Hutt (1961). Mortality among 7,000 pullets

following Roakin strain wing web vaccination in successive years was 3.2 and 7.2 per cent in strain K as compared to 0.7 and 0.8 per cent among strain C birds.

Finally, eradication of ND must be a constant goal. Restriction of movement of poultry, day-old chicks, and hatching eggs together with slaughter of infected birds and contacts, as have been practiced in Britain (1936 Fowl Pest Order), have been relied upon to achieve complete eradication (Reid, 1955). The occurrence of mild, largely subclinical forms of the malady, however, complicates the problem of its eventual total suppression.

Prevention by Sanitary Management

Basic specific precautions to exclude ND infection from the poultry operation and to prevent its spread include: For the hatchery—its strict isolation from broiler, dressing, or other poultry operations or plants; complete separation of buildings for brooding "started" chicks; separate labor for work outside of the hatchery; a policy of selling only day-old chicks; weekly inspection and production records on the supply flocks in order to permit exclusion of those showing a significant drop in egg production; exclusion of return of used chick boxes and feed sacks; proper disposal of hatchery wastes; and exclusion of visitors and nonessential personnel from the hatching and brooding areas. For the farm flock—exclusion of visitors, both bird and mammal; proper precautions in changing clothing and disinfecting footwear of the flock owner or caretaker after visiting possible sources of contamination; insisting on the practice of proper clothes-changing and disinfection precautions for blood-testing crews or other essential service personnel; buying replacement stock as day-old chicks from reliable hatcheries, preferably local, to minimize the chances of disease exposure during transit by means other than the personal vehicle of the buyer; rearing of the replacement flock on clean premises entirely apart from the adult flock, or of prior disposition of the

previous year's flock to maintain an all-pullet flock; preventing the return or introduction of used poultry crates, feed sacks, or other equipment, materials, or vehicles; proper manure disposal; proper disposition of dead birds by burning or deep burial; replacement of contaminated or unfit deep litter; and annual, or oftener, routine complete cleaning and disinfecting of the laying and brooder houses and range shelters. For the broiler plant—observation of the strict sanitary precautions already defined and the exclusion of birds from outside sources for slaughter or evisceration. For the produce plant—guarding against the purchase of poultry in the active stages of ND and thus the gross contamination of crates, vehicles, etc., which may carry the infection to birds in feeding stations, on farms, in hatcheries, and in broiler plants; frequent periodic cleaning and disinfection of facilities, equipment, and materials; and proper disposal of offal and wastes. For the feed dealer and processor—avoiding re-use of feed sacks unless properly cleaned and sterilized; practicing and encouraging proper measures to avoid spreading infection to and from farms. For the local veterinarian—disseminating knowledge on basic sanitation and advising the poultrymen on general and specific disease prevention problems; reaching an early diagnosis with prompt laboratory assistance and, if necessary, instituting a proper vaccination service and control program under the various circumstances encountered (Pomeroy and Brandly, 1953).

Experience with the extensive application of triethylene glycol aerosol as a means of controlling spread of ND in a large broiler plant led Ellis et al. (1952) to the conclusion that while the aerosol appeared to reduce the incidence of ND, as estimated by development of HI titers, it did not prevent the spread of benign ND infection or have a significant effect upon the weight of birds at slaughter. The hazards and means of sanitizing used burlap feed bags

contaminated with NDV and other agents have been clarified by Jungherr (1950).

Prevention by Vaccination

As with many viral diseases, experience with ND has stimulated efforts to minimize the shortcomings of both the killed or inactivated vaccines and the living, usually attenuated, vaccines (Beaudette, 1943, 1949, 1950, 1951; Beach, 1942, 1947; Traub, 1943–44; Brandly et al., 1946c; Hanson et al., 1951; Osteen et al., 1961; Jungherr and Markham, 1962; Lancaster, 1964; and others).

The necessity of more critical and satisfactory standards for commercial ND vaccines has prompted research and organization toward establishment of adequate criteria by various groups and agencies, especially in the U.S.A. (Technical Committee on Newcastle Disease of the North Central Region, Report of the Subcommittee on Vaccine Evaluation, 1951; Interregional Subcommittee on Vaccine Evaluation of the Advisory Committee on ND and Other Respiratory Diseases of Poultry —Johnson et al., 1954; Report Poultry Disease Subcommittee, 1959 and 1963). Major objectives have been provision of vaccine strains of maximal antigenicity and potency of suitably pathogenic challenge strains for assessment of immunity to various routes of exposure as well as of proper criteria for test birds and time of challenge for immunity after vaccination.

Tests applicable to identification and safety of vaccine strains of ND have been devised (Hanson and Brandly, 1955; Hanson, 1956; Lancaster, 1964). The need for rigid requirements of ND vaccine purity, especially with living virus vaccines, is emphasized by the demonstration of NDV in some lots of commercial pox and laryngotracheitis vaccines (Zargar and Pomeroy, 1950; Hofstad, 1949; and others) and of the possibility of vaccine contamination with lymphomatosis via the eggs of carrier hens used for ND vaccine production (Burmester et al., 1956).

Both types of Newcastle disease vaccines.

the inactivated virus and living virus, are now in general use. If properly prepared, handled, and administered, they may be expected to stimulate a substantial degree of immunity in a large proportion of healthy vaccinated fowl. However, even the more pathogenic living NDV vaccines fail to engender permanent or life-long protection in healthy, immunologically mature chickens against clinical or subclinical infection. At hatching, immunologic capacity is weak but strengthens substantially so as to equal that of the mature bird at about 6 weeks of age (Wolfe and Dilks, 1948). However, vaccination at an early age is frequently necessary where ND is enzootic (Ellis and Crook, 1952). Present knowledge regarding preparation, use, merits, and limitations of the two types may be summarized as follows:

Inactivated, or killed, Newcastle disease virus vaccines are prepared by growing suitably antigenic strains of virus in embryonating eggs, harvesting the dead or dying embryos and tissues, and inactivating the virus, usually by chemical agents, e.g., formaldehyde, crystal violet, beta-propriolactone. Growth of virus in tissue cultures for vaccine production is also being practiced. Adjuvants such as alumina gel are added to increase and prolong the immunizing effect. The vaccines must be tested for safety and potency and be given a "use expiration" date before release.

Each dose of the vaccine contains a relatively large quantity of killed virus, the normal reaction to which governs directly the degree and duration of the immunity which the bird can develop.

Inactivated Newcastle disease vaccine affords some protection by a blocking, or "interference," effect within several days to a week after injection. Specific immunity against Newcastle disease develops within a week after vaccination; it is well advanced after two weeks in healthy birds 10 days of age or older when vaccinated. All individuals in a flock may not develop a substantial immunity and the immunity may wane considerably 2 to 6 months after

vaccination. The degree to which protection is enhanced by revaccination depends upon the residual immunity, either active or passive, which is present at the time of revaccination. Critical work (Hofstad, 1953, 1954, 1955) has demonstrated that a minimal period of 9 weeks is required between initial vaccination and revaccination with killed vaccine if a maximal degree and duration of immunity to the "booster" dose is to be stimulated. Satisfactory reinforcement of immunity with living virus vaccine has been reported with intervals as short as 1 to 3 weeks (Geurden et al., 1950; Zuijdam, 1953; Lancaster, 1964).

The titer and persistence of immunity evoked by inactivated vaccines is usually less than that by the living vaccines, yet only the former may be relied upon to prevent undesirable effects of vaccination in laying flocks, in stock suffering from other diseases or devitalizing factors, and in baby chicks (Brandly, 1953; Van Roekel, 1955; Lancaster, 1964; Bankowski and Hill, 1954; Bankowski, 1961b; Gross, 1961; and others).

Living Newcastle disease virus vaccines. These are usually prepared by growing, in embryonating eggs, modified, or "weakened," strains of Newcastle disease virus (Komarov and Goldsmit, 1946; Brandly et al., 1946c; Van Roekel et al., 1948; Beaudette et al., 1949b; Clancy et al., 1949; Hitchner et al., 1950; Lancaster, 1964). Virus tissue cultures have also served as a source of ND vaccine (Bankowski and Boynton, 1948; Bankowski, 1950). Cultures of mammalian cells as a substitute for those of chicken origin are suggested in order to avoid contamination with chicken latent or orphan agents (Bankowski, 1957). Great care is required to keep the vaccine virus strain at a satisfactory stage of modification and to avoid contamination with other viruses and with bacteria. Usually the infected embryo material is dried to powder from the frozen state. Further refrigeration before it is used in reconstituted liquid or in dust form and proper care dur-

ing its use are required to keep the vaccine virus alive and capable of producing satisfactory results.

The living Newcastle disease vaccines now available from commercial sources are administered by different routes, including the "stick" or wing web puncture, intramuscular injection, "drop" intranasal or conjunctival sac installation, and by spraying or nebulizing as well as dusting for inhalation. Mechanical spraying of liquid vaccine and dispersion of micronized dry virus vaccine, as well as adding the virus to the drinking water, were designed to permit vaccination exposure and infection of entire lots or flocks, i.e., mass vaccination, thus saving the time and labor required for individual administration of the vaccine. The trend toward the mass method of vaccination, i.e., aerosol, dust or drinking water administration is indicated by the fact that about 91 per cent of the Newcastle disease either alone or combined with bronchitis vaccine produced in licensed establishments during the year ending June 30, 1964, was of this type (Agricultural Research Service, 1964). The saving of time, labor, and vaccine cost by mass vaccination is not without sacrifice in uniform and often stronger immunity resulting from vaccination of each bird of the flock (Lancaster, 1964).

Obvious disadvantages are lack of uniformity in dosage and particle size, particularly with very young and devitalized birds (underexposure and overexposure); great variation in environment (variable humidity and temperatures, foreign substances as well as viricidal factors in air and water); and likely exposure of persons and dissemination via air to other fowl and mammals (Bankowski and Hill, 1954; Markham et al., 1955, 1957; Van Waveren and Zuijdam, 1955; Johnson and Gross, 1952; Hitchner and Reising, 1953; Ceccarelli, 1954; Winterfield and Seadale, 1956; Larose and Van Roekel, 1959; Dardiri et al., 1962; Lancaster, 1964).

The NDV strains employed in living vaccines are of reduced or modified patho-

genicity. The least pathogenic (lentogenic) strains, e.g., B_1, La Sota, and F strains, are examples of those employed in birds of all ages for intranasal or intraocular instillation, admixture with the drinking water or dusting and spraying (Hanson and Brandly, 1955). The moderately pathogenic (mesogenic) strains, e.g., Roakin, MK 107 (L), Mukteswar, H (Hertfordshire), Haifa (Komarov), have been commonly used for wing web (intradermal) or intramuscular, or occasionally via feather follicle, vaccination of stock older than 4 weeks of nonlaying birds, and other disease-free, vigorous stock (Hanson and Brandly, 1955).

The quantity of virus introduced as living vaccine is so small that unless infection is established and multiplication ensues to a moderate degree, enough virus is not present to stimulate a satisfactory degree of immunity. The specific immunity engendered by living vaccine infection should appear within 5 to 7 days after vaccination and should be of substantial degree after the second week. The duration of immunity from living vaccine may vary greatly from flock to flock and among individuals. It may wane appreciably within 2 months, and proper revaccination is usually recommended within 2 months to a year (Doll et al., 1950c; Lancaster, 1964). However, revaccination of laying hens with mesogenic Roakin virus, within 1 to 2 weeks after initial vaccination with a killed vaccine, has been recommended (Zuijdam, 1953), and with the mesogenic Hertfordshire strain after 2 to 3 weeks (Geurden et al., 1950). One cannot, however, overlook the evidence that residual antibody, passively acquired (Brandly et al., 1946d) or resulting from vaccine or from natural infection, may impair the response to vaccination and revaccination even though less by respiratory than hypodermic or skin puncture introduction (Levine and Fabricant, 1950; Doll et al., 1950b, 1951; Markham et al., 1954; Winterfield and Seadale, 1957a; Bankowski et al., 1958; Keeble et al., 1963; Lancaster et al., 1960). Neverthe-

less, protective antibody concentration following vaccination or infection diminishes or is first lost by the epithelial lining of the respiratory tract and, hence, permits a "take" to inhalation revaccination earlier and more readily than by the deeper tissues which are in contact with higher antibody concentrations of the serum and tissue fluids. The dimensions of revaccination infection, reaction, and immunity "booster" effect are, therefore, directly dependent on the degree of antibody titer and tissue refractivity of the individual bird with proportional limitation of the degree and extent of the reinfection. Immunity of the respiratory epithelium to challenge infection does not become absolute in spite of excess dosages and repeated vaccination (Bankowski and Hill, 1954).

Adequate and reliable information on the maximal and average duration of a serviceable immunity to ND resulting from a single or repeated vaccination is limited (Lancaster, 1964). Age and individual immunologic capacity, as well as environmental and other factors affecting the host animal, all mitigate against uniformity of its immunity response to vaccination, especially under the great variations prevailing in the field. Inapparent infection from repeated exposure can be ascertained with reasonable but not unequivocal accuracy by testing for antibody titer before, and at intervals after, challenge. That failure to induce a lasting immunity is not characteristic of the less pathogenic strains of virus was illustrated with the use of a mesogenic Indian (Mukteswar) vaccine (Barnstein et al., 1949). These workers concluded in consequence of ND "breaks" following vaccination that the immunity engendered by it could not be depended upon for longer than a year. It is, however, necessary to differentiate between breaks due to antigenic inadequacy of the vaccine and those associated with improper handling and use of the vaccine (Jungherr and Markham, 1962; and others).

After initial live virus vaccination, the flock must be considered infected and capable of spreading the virus by excreta or eggs for three weeks or longer (Zuijdam, 1953); after re-exposure some birds may excrete virus for periods of three weeks (Markham et al., 1955). The possibilities that the vaccine virus may regain full activity and destructiveness are not great but cannot be entirely overlooked. ND vaccine virus used among six- to eight-week-old turkeys, without adverse effect, did spread and caused marked involvement as well as moderate mortality among two- to three-week-old poults on the same farm (Brandly et al., 1949).

Simultaneous vaccination against Newcastle disease and bronchitis or Newcastle disease and fowl pox has been further exploited toward saving labor and handling of birds. However, pox vaccination, either with chicken or pigeon source virus, requires manipulating the individual birds. Although a mixture of pigeon pox and ND viruses has been applied by the feather follicle method (Richter, 1956), an individual or mass method of ND vaccination may be applied immediately afterward to the birds comprising the group or flock.

The saving in time and expense of simultaneous vaccination invites the risk of interference with development of maximal immunity against one of the diseases (Hanson et al., 1956) or of exalting one of the agents (Hanson, 1957; and others). Also, there are various reports of the activation or aggravation of latent infections or parasitic effects and other devitalizing influences (Markham et al., 1957; Placidi, 1956; Russell, 1956).

It may not be expected that one kind or type of vaccine applied by a certain route or method will be adequate and satisfactory for all ages of birds and for various needs and circumstances. Factual, unbiased information free of commercial expediency together with judicious professional guidance are essential in the interests of the entire poultry industry.

Obviously the indiscriminate, careless,

nd improper use of vaccines will result in difficulty, confusion, and loss. Only when vaccination is practiced as an adjunct to a long-range sanitation and regulatory program, with eventual eradication of ND as the goal, can the control program progress effectively (Reid, 1955).

REFERENCES

Ackermann, W. Wilbur: 1964. Cell surface phenomena of Newcastle disease virus, p. 153. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

Adler, H. E., Willers, E. H., and Campbell, J.: 1951. Newcastle disease (avian pneumoencephalitis) in Hawaii. Am. Jour. Vet. Res. 12:44.

Agricultural Research Service, USDA.: 1964. Biological Products Notice 128, Activities of Licensed Establishments Supervised by the Animal Inspection and Quarantine Division.

Albiston, H. E., and Gorrie, J. R.: 1942. Newcastle disease in Victoria. Australian Vet. Jour. 18:75.

André, J., and Audebaud, C.: 1960. Recherches sur le culture du virus de Newcastle et sur ses applications pratiques. Ann. Inst. Past. 98:829.

Anonymous: 1948. Newcastle disease in poultry. Vet. Jour. 104:273.

Asdell, Mary K., and Hanson, R. P.: 1960. Sequential changes in the titer of Newcastle disease virus in tissues—a measure of the defense mechanisms of the chicken. Am Jour. Vet. Res. 21:128.

Asplin, F. D.: 1947. Newcastle disease in ducks and geese. Vet. Record 59:621.

————: 1952. Immunization against Newcastle disease with a virus of low virulence (Strain F) and observations on sub-clinical infection in partially resistant fowls. Vet. Record 64:245.

Bacrynski, Z.: 1959. [Carriers of Newcastle disease.] I. Rats and mice.] Abst. in Vet. Bul. 29:623.

Bang, F. B.: 1948. Filamentous forms of Newcastle virus. Proc. Soc. Exper. Biol. Med. 68:5.

————: 1948. Studies on Newcastle disease virus. I. An evaluation of the method of titration. Jour. Exper. Med. 88:233.

————: 1952. Development of Newcastle disease virus in chick embryo cells. A study with the electron microscope. Proc. Am. Soc. Exper. Biol. 11:408.

————: 1964. Pathogenesis in the embryo, p. 247. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

————, and Foard, M.: 1953. Variables in a virus neutralization test. Newcastle disease in the chick embryo. Proc. Soc. Exper. Biol. 15:486.

————, and Foard, M.: 1956. The serology of Newcastle disease virus infection. I. The reaction between various sera and the virus. II. The antigenic relationships of Newcastle virus. III. Prevalence of antibody against Newcastle in individuals closely exposed to the virus and in the absence of conjunctivitis. Jour. Immunol. 76-342.

Bankowski, R. A.: 1946. Cited by J. R. Beach in Biester and Schwarte, Diseases of Poultry, p. 489, 1947. Iowa State College Press, Ames.

————: 1950. Further studies on in vitro cultivated pneumoencephalitis (Newcastle disease) virus and its use as a vaccine. Vet. Med. 45:322.

————: 1957. A modified live Newcastle disease virus vaccine. Proc. Soc. Exper. Biol. and Med. 96:114.

————: 1961a. A study of asymptomatic Newcastle disease in a breeding flock. Res. Vet. Sci. 2:193.

————: 1961b. Respiratory disease complex of chickens in the United States. Br. Vet. Jour. 6:117.

————: 1964. Cytopathogenicity of Newcastle disease virus, p. 231. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

————, and Boynton, W. H.: 1948. Preliminary report on the propagation of avian pneumoencephalitis virus (Newcastle disease) in vitro. Vet. Med. 43:505.

————, and Corstvet, R.: 1961. Isolation of hemagglutinating agent distinct from Newcastle disease from the respiratory tract of chickens. Avian Dis. 5:253.

————, Corstvet, R., and Fabricant, J.: 1958. A tissue culture-modified Newcastle disease virus II. Immunogenicity of the tissue culture-modified Newcastle disease virus in chickens. Avian Dis. 2:227.

————, and Hill, R. W.: 1954. Factors influencing the efficiency of vaccination of chickens against Newcastle disease by the air borne route. Proc. 91st Ann. Meet. Am. Vet. Med. Assn., p. 317.

————, Irawa, H., and Hyde, J.: 1959. Tissue culture—a diagnostic tool—with particular reference to Newcastle disease and vesicular exanthema viruses. Proc. 63rd Meet. U.S. Livestock Sanit. Assn., p. 377.

Barber, C.: 1947. Newcastle disease diagnosed in Georgia. Cornell Vet. 37:260.

Baron, Samuel: 1964. Relationship of interferon and temperature to virulence of Newcastle disease virus, p. 205. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

Baskaya, H., Burd, H. E., Hudson, C. B., and Bivins, J. A.: 1952. A comparison of Newcastle disease virus recovery from bone marrow and from pools of respiratory tract and spleen. Am. Jour. Vet. Res. 13:405.

Beach, J. R.: 1942. Avian pneumoencephalitis. Proc. 46th Ann. Meet. U.S. Livestock Sanit. Assn., p. 203.

———: 1944. The neutralization in vitro of avian pneumoencephalitis virus by Newcastle disease immune serum. Science 100:361.

———: 1947. Chapter on pneumoencephalitis (ND) in Biester and Schwarte, Diseases of Poultry. 1947. Iowa State College Press, Ames.

———: 1948. The application of the hemagglutination-inhibition test in the diagnosis of avian pneumoencephalitis (Newcastle disease). Jour. Am. Vet. Med. Assn. 112:85.

Beamer, P. D., and Prier, J. E.: 1950. Studies on Newcastle disease. III. Resistance of Newcastle disease virus to certain chemical agents. Cornell Vet. 40.56.

Beaudette, F. R.: 1943. A review of the literature on Newcastle disease. Proc. 47th Ann. Meet. U.S. Livestock Sanit. Assn., p. 122.

———: 1948. The immunization of birds against Newcastle disease. Proc. 52nd Ann. Meet. U.S. Livestock Sanit. Assn., p. 254.

———: 1949. An addendum to a review of the literature on Newcastle disease. Proc. 53rd Ann. Meet. U.S. Livestock Sanit. Assn., p. 202.

———: 1950. Recent literature on Newcastle disease. Proc. 54th Ann. Meet. U.S. Livestock Sanit. Assn., p. 132.

———: 1951. Current literature on Newcastle disease. Proc. 55th Ann. Meet. U.S. Livestock Sanit. Assn., p. 108.

———: 1952. Personal communication.

———, Bivins, J. A., and Miller, B. R.: 1948. Use of antibiotic agents for bacterial sterilization of respiratory exudates from naturally infected cases of Newcastle disease. Am. Jour. Vet. Res. 9.97.

———, Bivins, J. A., and Miller, B. R.: 1949a. A comparison of filtration and antibiotic treatment for the recovery of Newcastle virus from spontaneous cases. Am. Jour. Vet. Res. 10.92.

———, Bivins, J. A., and Miller, B. R.: 1949b. Newcastle disease immunization with live virus. Cornell Vet. 39:302.

———, and Hudson, C. B.: 1956. Evidence of Newcastle disease in the eastern United States as early as 1938. Cornell Vet. 46.227.

Berg, L. R., Bearse, G. E., and Hamilton, C. M.: 1947. The effect of Newcastle disease on egg production and egg quality. Poultry Sci. 26.614.

Binns, W., Nielsen, H. M., and Miner, M. L.: 1949. Severe Newcastle disease outbreak causes serious losses to Utah poultry industry. Farm and Home Sci. 10:17.

Biswal, G.: 1954. Additional histological findings in the chicken reproductive tract. Poultry Sci. 33.845.

———, and Morrill, C. C.: 1954. The pathology of the reproductive tract of laying pullets affected with Newcastle disease. Poultry Sci. 33:880.

Blattner, R. J., and Williamson, A. P.: 1951. Developmental abnormalities in the chick embryo following infection with Newcastle disease virus. Proc. Soc. Exper. Biol. Med. 77:619.

Blood, B.D.: 1950. Epidemiology of Newcastle disease. Bul. Pan Am. Sanit. Bur. 29.28.

Bolin, F. M.: 1948. Isolation of Newcastle disease virus from feces of the domestic cat and the common chicken louse. Proc. 48th Ann. Meet. Soc. Am. Bacteriologists. P. 43.

Boney, W. A.: 1951. The isolation of a neurotropic strain (GB) of Newcastle disease virus. Southwestern Vet. 5:19.

Bornstein, S., Rautenstein, A., and Moses, E.: 1949. A large-scale vaccination breakdown with "Mukteswar" Newcastle vaccine, and its investigation by means of the haemagglutination inhibition test. Refuah Vet. 6:158.

Boulanger, P., and Rice, C. E.: 1953. A study of complement-fixation methods as applied to the demonstration of antibodies in birds. Proc. Am. Vet. Med. Assn., p. 316.

Bower, R. K., and Eisenstark, A.: 1954. The effects of certain chemical agents upon Newcastle disease virus with special reference to the action of urethane. Kans. Acad. Sci. Trans. 57:291.

Boyd, R. J., and Hanson, R. P.: 1958. Survival of Newcastle disease virus in nature. Avian Dis. 2:83.

Brandly, C. A.: 1945. Unpublished data.

———: 1947. Report of committee on transmissible diseases of poultry. Proc. 52nd Ann. Meet. U.S. Livestock Sanit. Assn., p. 312.

———: 1948. Unpublished data.

———: 1950. Newcastle disease. Jour. Am. Vet. Med. Assn. 116 139.

———: 1951. Poultry diseases as public health problems. Pub. Health Rep. 66.668.

———: 1953. Epizootiology of Newcastle disease. Proc. 15th Internat. Vet. Cong. Stockholm 1:233.

———, Hanson, R. P., and Hoyt, H. H.: 1949. Unpublished data.

———, Hanson, R. P., Lewis, S. H., Winslow, N. S., Pritchard, W. R., Hoyt, H. H., and Nerlinger, C. M.: 1947. Variables and correlations in laboratory procedures for Newcastle disease diagnosis. Cornell Vet. 37:324.

———, Moses, H. E., and Jones, J. E.: 1944. Special report from the Huntington Laboratory to the War Dept., March 27, 1944.

———, Moses, H. E., Jones, E. E., and Jungherr, E. L.: 1946a. Epizootiology of Newcastle disease of poultry. Am. Jour. Vet. Res. 7:245.

———, Moses, H. E., Jungherr, E. L., and Jones, E. E.: 1946b. The isolation and identification of Newcastle disease virus. Am. Jour. Vet. Res. 7:289.

———, Moses, H. E., Jones, E. E., and Jungherr, E. L.: 1946c. Immunization of chickens against Newcastle disease. Am. Jour. Vet. Res. 7:307.

———, Moses, H. E., and Jungherr, E. L.: 1946d. Transmission of antiviral activity via the egg and the role of congenital passive immunity to Newcastle disease in chickens. Am. Jour. Vet. Res. 7:333.

Brandt, C. D.: 1961. Cytopathic action of myxoviruses on cultivated mammalian cells. Virology 14:1.

Brion, A., and Fontaine, M.: 1960. Maladie de Newcastle problemes actuels. Acad. Vet. de France Bul. 33:219.

Brueckner, A. L., Reagan, R. L., Schenck, D. M., Werner, H. O., and Hickman, J. W.: 1950. Mammalian adaptations of Newcastle disease virus. Proc. Soc. Exp. Med. Assn., p. 163.

Brumfield, H. P., and Pomeroy, B. S.: 1957. Direct complement fixation by turkey and chicken serum in viral systems. Proc. Soc. Exp. Biol. 94:146.

Buddingh, G. J.: 1952. The pathological effects of viruses on the chick embryo. Ann. N.Y. Acad. Sci. 55:248.

Bureau of Animal Industry: 1946a. The diagnosis of Newcastle disease. Bur. Anim. Ind. U.S. Dept. Agr. Bul., Aug. 15.

———: 1946b. The hemagglutination and hemagglutination-inhibition tests for the diagnosis of Newcastle disease. Bur. Anim. Ind. U.S. Dept. Agr. Bul., Oct. 21.

Burmester, B. R., Cunningham, C. H., Cottral, G. E., Belding, R. C., and Gentry, R. F.: 1956. The transmission of visceral lymphomatosis with the virus Newcastle disease vaccines. Am. Jour. Vet. Res. 17:283.

Burnet, F. M.: 1942. The affinity of Newcastle disease virus to the influenza virus group. Australian Jour. Exper. Biol. Med. Sci. 20:81.

———: 1943. Human infection with virus of Newcastle disease of fowls. Med. Jour. Australia 2:313.

———: 1950. The haemolytic action of Newcastle disease virus. I. Two types of interaction between virus and red cells. Australian Jour. Exper. Biol. Med. Sci. 28:299.

——— and Ferry, J. D.: 1934. The differentiation of the viruses of fowl plague and Newcastle disease: experiments using the technique of chorio-allantoic membrane inoculation of the developing egg. Brit. Jour. Exper. Path. 15:56.

——— and Lind, P. E.: 1950. Haemolysis by Newcastle disease virus. II. General character of the haemolytic action. Australian Jour. Exper. Biol. Med. Sci. 28:129.

Byerly, T. C.: 1948. Report of the committee on incidence of Newcastle disease. Jour. Am. Vet. Med. Assn. 12:125.

Cairns, H. J. F.: 1951. The growth of influenza viruses and Newcastle disease virus in mouse brain. Brit. Jour. Exper. Path. 32:110.

Ceccarelli, A.: 1954. Il Vaccino "H" (ceppo Hertsfordshire) nella profilassi della pseudopeste aviare. Zooprofilassi 9.421.

Chanock, R. M.: 1955. Cytopathogenic effect of Newcastle disease virus in monkey kidney cultures and interference with poliomyelitis viruses. Proc. Soc. Exper. Biol. 89.379.

———, and Coates, H. V.: 1961. Myxoviruses—A comparative description, p. 279. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

Chu, H. P.: 1953. The agglutination of spermatozoa by viruses in influenza, mumps and Newcastle disease. Proc. 6th Intern. Cong. Microbiol. 20.

Chute, H. L., and O'Meara, D. C.: 1963. The development of chickens free of common poultry disease vaccines. Maine Agr. Exper. Sta. Bul. 613.

Clancy, C. F., Cox, H. R., and Bottorff, C. A.: 1949. Laboratory experiments with living Newcastle disease vaccine. Poultry Sci. 28:58.

Clark, D. S., Jones, J. E., and Ross, F. K.: 1955. The use of aqueous humor for early diagnosis of Newcastle disease. Am. Jour. Vet. Res. 16:158.

———, Jones, E. E., and Ross, F. K.: 1957. Further studies with the aqueous humor of chickens as a reservoir for the virus of Newcastle disease. Am. Jour. Vet. Res. 18:204.

Clark, E., and Nagler, F. P. O.: 1943. Haemagglutination by viruses. The range of susceptible cells with special reference to agglutination by vaccinia virus. Australian Jour. Exper. Biol. Med. Sci. 21:103.

Clegg, R. E., and Mueller, C. D.: 1951. Calcium metabolism during Newcastle disease. Poultry Sci. 30:157.

Cole, R. K., and Hutt, F. B.: 1961. Genetic differences in resistance to Newcastle disease. Avian Dis. 5:205.

Coleman, P. H.: 1959. Studies of the antigenic nature of the myxoviruses. Univ. of Wis. doctoral thesis.

Coronel, A. B.: 1947. Newcastle disease in the Philippines with special reference to immunization. Trop. Vet. Med. 10:1566.

Crawford, M.: 1950. Ranikhet Disease. Ann. Rep. Gov. Vet. Surgeon. Colombo, Ceylon.

Crawley, J. F.: 1954. Immunization of chickens against infectious bronchitis and Newcastle disease by the spray method. Proc. 10th World's Poultry Cong. Edinburgh 2:254.

Crook, E.: 1951. Unpublished data.

Cunningham, C. H.: 1948. The effect of certain chemical agents on the virus of Newcastle disease of chickens. Am. Jour. Vet. Res. 9:195.

———: 1952. Methods employed in the diagnosis and investigation of infectious bronchitis and Newcastle disease. Proc. Am. Vet. Med. Assn., p. 250.

———: 1965. Newcastle disease. Chapt. 15. In Thos. G. Hull (ed.), Diseases Transmitted from Animals to Man, 5th ed., Charles C. Thomas, Springfield, Ill.

Dardiri, A. H., Yates, V. J., and Flanagan, T. D.: 1962. The reaction to infection with the B-1 strain of Newcastle disease in man. Am. Jour. Vet. Res. 23.918.

Davis, C. R., Moulthrop, I. M., and Reagan, R. L.: 1950. Laboratory and field studies of Newcastle disease vaccines. Proc. 87th Meet. Am. Vet. Med. Assn. p. 291.

Delaplane, J. P., and Stuart, H. C.: 1943. The propagation of a virus in embryonated chicken eggs causing a chronic respiratory disease of chickens. Am. Jour. Vet. Res. 4:325.

DeLay, P. D.: 1947. Isolation of avian pneumoencephalitis (Newcastle disease) virus from the yolk sac of day-old chickens, embryos, and infertile eggs. Science 106:545.

———, DeOme, R. B., and Bankowski, R. A.: 1948. Recovery of pneumoencephalitis (Newcastle) virus from the air of chicken houses containing infected birds. Science 107:474.

Dinter, Z.: 1964. Avian myxoviruses, p. 299. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

———, and Bakos, K.: 1935. Über die Ausscheidung des Virus der Newcastle-Krankheit nach der Testinfektion immuner Huhner. Arch. Exper. Vet. Med. 7 514.

———, Hermodsson, S., and Hermodsson, L.: 1964. Studies on myxovirus Yucaipa: its classification as a member of the paramyxovirus group. Virology 22:297.

Divo, A.: 1950. La enfermedad de Newcastle (Neunoencefalitis aviaria) en Venezuela. Venezuela Inst. Invest. Vet. Bol. 3:547.

———: 1961. Cited by Monteverde et al., 1962.

Dobson, N: 1959. Newcastle disease. Proc. Seventh World's Poultry Cong., p. 250.

———, and Simmons, G. C.: 1951. The introduction of Newcastle disease by means of frozen poultry carcasses. Off. Rep. 9th World's Poultry Cong., Paris 3:18.

Doll, E. R., Wallace, M. E., and McCollum, W. H.: 1950a. Preincubation Inoculation of eggs with Newcastle disease virus. Poultry Sci. 29:582.

———, Wallace, M. E., and McCollum, W. H.: 1950b. Interpretation of serologic procedures for the diagnosis of Newcastle disease. Am. Jour. Vet. Res. 11:265.

———, Wallace, M. E., and McCollum, W. H.: 1950c. Reinfection of chickens vaccinated by the intranasal method with live B1 Newcastle disease virus. Am. Jour. Vet. Res. 11:437.

———, McCollum, W. H., and Wallace, M. E.: 1951. Susceptibility to Newcastle disease infection of chickens from herd immunized with live virus vaccines. Am. Jour. Vet. Res. 12:232.

Doyle, T. M.: 1927. A hitherto unrecorded disease of fowls due to a filter-passing virus. Jour. Comp. Path. 40.144.

———: 1935. The virus diseases of animals with special reference to those of poultry. Jour. Comp. Path. 46:90.

Ellis, C. C., and Crook, E.: 1952. Sanitation and vaccination in the control of Newcastle and other diseases in a large broiler plant. Proc. 17.5. Livestock Sanit. Assn. 56:284.

Ellis, P., Brandly, C. A., and Hanson, R. P.: 1952. The influence of triethylene glycol aerosol on the growth, morbidity and mortality rates of a broiler flock. Poultry Sci. 31:394.

Evans, A. S.: 1955. Pathogenicity and immunology of Newcastle disease virus in man. Am. Jour. Pub. Health 45:742.

———: 1956. The laboratory diagnosis of Newcastle disease in man. Am. Jour. Clin. Path. 26:163.

Evans, C. A., and Melnick, D. L.: 1950. Attempts to produce lymphocytopenia in rabbits following intravenous inoculation of certain viruses. Jour. Infect. Dis. 86:223.

Fabry, J. E., and Crawley, J. F.: 1954. Studies on chronic respiratory disease of chickens. III. Egg transmission of a pleuropneumonia-like organism. Canad. Jour. Comp. Med. 18.67.

Flanagan, A. D., Love, R., and Tesar, W.: 1955. Propagation of Newcastle disease virus in Ehrlich ascites cells in vitro and in vivo. Proc. Soc. Exper. Biol. Med. 90 82.

Foster, N. M., and Thompson, C. H., Jr.: 1957. The comparative thermolability of four strains of Newcastle disease virus of widely varying virulence. Vet. Med. 52:119.

Francis, D. W., and Kish, A. F.: 1955. Familial resistance to Newcastle disease in a strain of New Hampshires. Poultry Sci. 34:531.

French, E. L.: 1952. The pyrogenic effect of the influenza mumps group of viruses in the laboratory rabbit. Australian Jour. Exper. Biol. Med. 30:479.

Freyman, M. W., and Bang, F. B.: 1949. Human conjunctivitis due to Newcastle virus in the U.S.A. Johns Hopkins Hosp. Bul. 84:409.

Gardner, E., Jr., Wallace, J. H., Dodd, M. C., and Wright, C.S.: 1954. Antigenically modified red cells in chickens infected with Newcastle disease. Proc. Soc. Exper. Biol. 87:253.

Gelencei, E., and Bordt, D.: 1960. Studies of Newcastle disease virus strains in various cell cultures. Am. Jour. Vet. Res. 21:987.

Geuzten, L. M. G., and Devos, A.: 1955. Laboratoriumsdiagnose der atypischen Geflügelpest. Wien. tierärztl. Mschr. 42:65.

————, Devos, A., and Mortelmans, J.: 1950. Immunisatieproeven tegen Pseudovogelpest. Vlaams diergeneesk. Tijdschr. 19:177.

Gillespie, J. H., Kessel, B., and Fabricant, J.: 1950. The isolation of Newcastle disease virus from a starling. Cornell Vet. 40:93.

Giltner, L. T.: 1950. Cited by Blood, 1950.

Ginsberg, H. D.: 1951. Mechanics of production of pulmonary lesions in mice by Newcastle disease virus. Jour. Exper. Med. 94:191.

Goldhaft, T. M., and Wernicoff, N.: 1948. High mortality associated with a widespread outbreak of Newcastle disease. Cornell Vet. 38:181.

Goldman, E. C., and Hanson, R. P.: 1955. The isolation and characterization of heat-resistant mutants of the Najarian strain of NDV. Jour. Immunol. 74:101.

Goldwasser, R., and Kohn, A.: 1957. Neutralization and titration of Newcastle disease virus in chicken embryo tissue cultures. Am. Jour. Vet. Res. 18:890.

Gordon, R. F., and Asplin, F. D.: 1952. Newcastle disease in England and Wales. Vet. Record 59:197.

————, Reid, J., and Asplin, F. D.: 1948. Newcastle disease in England and Wales. Proc. Eighth World's Poultry Cong., p. 642.

Granoff, A.: 1962. Heterogensis and phenotypic mixing with Newcastle disease virus. Cold Spring Harbor Symposia. Quant. Biol. 27:519.

————: 1964. Nature of the Newcastle disease virus population, p. 107, in R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

————, and Henle, W.: 1954. Studies on the hemolytic activity of Newcastle disease virus (NDV). Jour. Immunol. 72:322.

————, and Hirst, G. K.: 1954. Experimental production of combination forms of virus. IV. Mixed influenza A-Newcastle disease virus infections. Proc. Soc. Exper. Biol. 86:84.

Gray, J. J., Snoeyenbos, G. H., and Peck, H. A.: 1954. Newcastle disease in turkeys. Report of a field outbreak. Jour. Am. Vet. Med. Assn. 124:302.

Gross, W. B.: 1961. Escherichia coli as a complicating factor in Newcastle disease vaccination. Avian Dis. 5:132.

Groupe, V., and Dougherty, R. M.: 1956. Neuropathic effect of Newcastle disease virus in mice and modification of host response by receptor destroying enzyme, viral interference, and xerosin. Jour. Immunol. 76:130.

Gustafson, D. P., and Moses, H. E.: 1953. The English sparrow as a natural carrier of Newcastle disease virus. Am. Jour. Vet. Res. 14:581.

Hanson, L. E.: 1954. Separation of Newcastle disease and infectious bronchitis viruses in mixed infections. Poultry Sci. 33:223.

————: 1957. Some factors responsible for variations in viral immunity. Jour. Am. Vet. Med. Assn. 130:505.

————, White, F. H., and Alberts, J. O.: 1956. Interference between Newcastle disease and infectious bronchitis viruses. Am. Jour. Vet. Res. 17:294.

Hanson, R. P.: 1949. Characteristics of certain strains of Newcastle disease virus. Univ. of Wis. doctoral thesis.

————: 1953. Personal communication.

————: 1956. An intercerebral inoculation test for determining the safety of Newcastle disease vaccines. Am. Jour. Vet. Res. 17:16.

————, and Brandly, C. A.: 1955. Identification of vaccine strains of Newcastle disease virus. Science 122:156.

————, and Brandly, C. A.: 1958. Newcastle disease. Symposium on animal disease and human health. Ann. N.Y. Acad. Sci. 70:585.

————, Crook, E., and Brandly, C. A.: 1951. Comparisons of immunogenicity of five strains of Newcastle disease virus as formaulitized vaccines. Vet. Med. 46:451.

————, and Sinha, S. K.: 1952. Epizootic of Newcastle disease in pigeons and studies on transmission of the virus. Poultry Sci. 31:404.

————, Upton, E., and Brandly, C. A.: 1951. Pneumopathogenicity of Newcastle disease virus for adult white mice. Jour. Bact. 62:545.

————, Upton, E., Brandly, C. A., and Winslow, N. S.: 1949. Heat stability of hemagglutinin of various strains of Newcastle disease virus. Proc. Soc. Exper. Biol. Med. 70.283.

Hanson, R. P., Winslow, N. S., and Brandly, C. A.: 1947. Influence of the route of inoculation of Newcastle disease virus on selective infection of the embryonating egg. Am. Jour. Vet. Res. 8:416.

———, Winslow, N.S., Brandly, C. A., and Upton, E.: 1950. Antiviral activity of Newcastle disease immune sera. Jour. Bact. 60:557.

Hartwigk, H., and Ansch, G.: 1957. Zur Frage der Empfänglichkeit von Sperlingen für atypische Geflügelpest (Newcastle disease). Berliner Münchener tierärztl. Wochenschr. 70:285.

Hirst, G. K., and Pickels, E. G.: 1942. A method for the titration of influenza hemagglutinins and influenza antibodies with the aid of a photo elective densitometer. Jour. Immunol. 45:273.

Hitchner, S. B., and Reising, G.: 1953. Results of field tests on spraying a commercially prepared Newcastle disease vaccine. Proc. 90th Ann. Meet. Am. Vet. Med. Assn., p. 350.

———, Reising, G., and Van Roeckel, H.: 1950. The intranasal vaccine—its role in a Newcastle disease control program. Proc. 54th Ann. Meet. U.S. Livestock Sanit. Assn., p. 154.

Hofstad, M. S.: 1948. Personal communication.

———: 1949a. Recovery of Newcastle disease (pneumoencephalitis) virus from mites, Liponyssus sylvarium, after feeding upon Newcastle infected chickens. Am. Jour. Vet. Res. 10.570.

———: 1949b. A study on the epizootiology of Newcastle disease (pneumoencephalitis), Poultry Sci. 28:530.

———: 1950. Experimental inoculation of swine and sheep with Newcastle disease virus. Cornell Vet. 40:190.

———: 1951. A quantitative study of Newcastle disease virus in tissues of infected chickens. Am. Jour. Vet. Res. 12:334.

———: 1953. Immunization of chickens against Newcastle disease by formalin-inactivated virus. Am. Jour. Vet. Res. 14:586.

———: 1954. The secondary immune response in chickens revaccinated with inactivated Newcastle disease virus vaccine. Am. Jour. Vet. Res. 15:604.

———: 1955. The immune response in chickens following the use of three different types of inactivated Newcastle disease vaccine. Am. Jour. Vet. Res. 16.608.

Ingalls, W. L., Vesper, R. W., and Mahoney, A.: 1951. Isolation of Newcastle disease virus from the great horned owl. Jour. Am. Vet. Med. Assn. 119.71.

Iyer, S. G., and Dobson, N.: 1940. A successful method of immunization against Newcastle disease of fowls. Vet. Record 52.889.

Jacotot, H., Vallée, A., and Le Priol, A.: 1955. Réédive, après quatre ans et demi, d'une conjunctivite humaine à virus de Newcastle. Ann. Inst. Past. 88:111.

Jakubik, J.: 1962. [Use of tissue culture for the diagnosis of Newcastle disease.] Abstr. in Vet. Bul. 32, no. 3738.

Jansen, J., Kunst, H., Van Dorssen, C. A., and Vander Berg, H. A.: 1949. Pseudovogelpest bij Fazanten uit Calcutta. Tijdschr. v. Diesgeneesk. 74:533.

Johnson, E. P., and Gross, W. B.: 1952. Vaccination against pneumoencephalitis (Newcastle disease) by atomization or nebulization in incubators and chick boxes with the B₁ virus. Am. Vet. Med. 47:364.

———, Hanson, R. P., Rosenwald, A. S., and Van Roeckel, H.: 1954. The responsibility of state and federal agencies in the improvement of poultry vaccines. Jour. Am. Vet. Med. Assn. 125:441.

Johnstone, R. N.: 1933. Pseudo poultry plague. The second outbreak. Victoria, Australia. Dept. Agr. Jour. 31:80.

Junghern, E. L.: 1946. Proc. Conference on Newcastle disease. U.S. Dept. of Agr., May 2, 3, p. 93.

———: 1948. Report of the Committee on modes of spread of Newcastle disease. Jour. Am. Vet. Med. Assn. 112:124.

———: 1950. Studies on sanitizing used feed bags. Jour. Am. Vet. Med. Assn. 117.324.

———, Brandly, C. A., and Moses, H. E.: 1945. Unpublished data.

———, and Markham, F. S.: 1962. Relationship between a Puerto Rican epizootic and the B-1 strain of Newcastle disease virus. Poultry Sci. 41.522.

———, and Terrell, N.: 1946. Observations on the spread of Newcastle disease. Proc. 50th Ann. Meet. U.S. Livestock Sanit. Assn., p. 158.

———, Tyzzer, J. E., Brandly, C. A., and Moses, H. E.: 1946. The comparative pathology of fowl plague and Newcastle disease. Am. Jour. Vet. Res. 7:250.

Kaplan, M.: 1949. Newcastle disease. Some important animal diseases in Europe. FAO Agr. Studies, United Nations 10:149.

Karzon, D. T.: 1956. Non-specific viral inactivating substance (VIS) in human and mammalian sera. Natural antagonists to the inactivator of Newcastle disease virus and observations on the nature of the union between the inactivator and virus. Jour. Immunol. 76:454.

———, and Bang, F. B.: 1951. The pathogenesis of infection with a virulent (GC 179) and an avirulent (B) strain of Newcastle disease in the chicken. I. Comparative rates of viral multiplication. Jour. Exper. Med. 93:267.

Kaschula, V. R.: 1950. The epizootiology of Newcastle disease and its control by vaccination. Jour. South Africa Vet. Med. Assn. 21:154.

————: 1961a. A comparison of disease in village and in modern poultry flocks in Nigeria. Bul. Epiz. Dis. Africa 9:397.

————: 1961b. The pattern of distribution of lesions in Newcastle disease in Northern Nigeria. Jour. Comp. Path. and Therap. 71:343.

Kawashima, H., Sato, T., and Hanaki, T.: 1953. The latest outbreak of Newcastle disease in Japan. Exper. Rep. Govt. Exper. Sta. Anim. Hyg., Japan 27:151.

Keeble, S. A., Box, P. G., and Christie, D. W.: 1963. Vaccination against Newcastle disease. Vet. Record 75:151.

Keeney, A. H., and Hunter, M. C.: 1950. Human infection with the Newcastle virus of fowls. Am. Med Assn. Arch. Ophthal. 44:573.

Keymer, I. F.: 1961. Newcastle disease in the jackdaw (Corvus monedula) Vet. Record 73:119.

Kilham, L.: 1949. A Newcastle disease virus (NDV) hemolysin. Proc. Soc. Exper. Biol. 71:63.

————, Jungherr, E. L., and Luginbuhl, R. E.: 1949. Antihemagglutinating and neutralizing factors against Newcastle disease virus occurring in sera of patients convalescent from mumps. Jour. Immunol. 63:37.

Kingsbury, D. W.: 1962. Use of actinomycin D to unmask RNA synthesis induced by Newcastle disease virus. Biochem. and Biophys Res. Commun. 9(½):156.

Knox, C. W.: 1950. The effect of Newcastle disease on egg production, egg weight and mortality rate. Poultry Sci. 29:907.

Kohn, A.: 1955. Quantitative aspects of Newcastle disease virus infection—effect of route of infection on the susceptibility of chicks. Am. Jour. Vet. Res. 16:450.

————: 1959. The role of the alimentary tract and the spleen in Newcastle disease. Am. Jour. Hygiene 69:167.

Komarov, A.: 1940. Newcastle disease in Palestine. Palestine Vet. Bul. 6:107.

————: 1947. Personal communication.

————, and Goldsmit, L.: 1946. Preliminary observation on the modification of a strain of Newcastle disease virus by intracerebral passage through ducklings. Vet. Jour. 102:212.

Koser, A.: 1942. Der derzeitige Geflugelpest Seuchenzug. Berliner Munch. tierarztl. Wochenschr. 50:446.

Kraneveld, F. C.: 1926. Over een in Ned-Indie heerschende Ziekte onder het Pluimvee. Nederland Ind. Bladen Diergeneesk. 38:448.

————, and Mansjoer, M.: 1950. Twee Mogelijkheden tot Besmetting van pluimveebedrijven met Pseudovogelpest (Swage and Newcastle disease). Hemera Zoa. Dl. 57:166.

Lancaster, J. E.: 1964. Newcastle disease—Control by vaccination. Vet. Bul. 34:57.

————, Merriman, M., and Reinis, A. A.: 1960. The intranasal Newcastle disease vaccination of chicks from immune parents. Canad. Jour. Comp. Med. 24:52.

Larose, R. N., and Van Roekel, H.: 1959. Response of chicken flocks to commercial Newcastle disease and infectious bronchitis vaccines. Poultry Sci. 38:1221.

Levine, P. P.: 1962. The place of vaccination in the control of poultry diseases. Vet. Rec. 74:1394.

————, and Fabricant, J.: 1950. Susceptibility to Newcastle infection of chicks with congenital serum antibodies. Cornell Vet. 40:213.

————, and Fabricant, J.: 1952. Efficacy of Newcastle disease vaccines under controlled conditions. Cornell Vet. 42:449.

————, Fabricant, J., Gillespie, J. H., Angstrom, C. I., and Mitchell, G. D.: 1950. The results of pen contact exposure of susceptible chickens to chickens recovered from Newcastle disease. Cornell Vet. 40:206.

Levine, S., and Sagik, B. P.: 1956. The interactions of Newcastle disease virus (NDV) with chick embryo tissue culture cells: attachment and growth. Virology 2.57.

Liao, V. S.: 1951. Studies on the nature of Newcastle disease virus. Doctoral thesis, Kans. St. Coll. P. 89.

Linn, H. D.: 1948. Uniform interstate laws and regulations. Proc. 52nd Ann. Meet. U.S. Livestock Sanit. Assn., p. 215.

Lippmann, O.: 1952. Human conjunctivitis due to the Newcastle disease virus of fowls. Am. Jour. Ophthal. 35:1021.

Lissot, G.: 1956. Peste aviaire, varieté maladie de Newcastle, à virus faible. Bul. Acad. vét. France 29:43, 45.

Lorenz, F. W., and Newlon, W. E.: 1944. Influence of avian pneumoencephalitis on subsequent egg quality. Poultry Sci. 23:193.

Luginbuhl, R. E., and Jungherr, E.: 1949. A plate hemagglutination-inhibition test for Newcastle disease antibodies in avian and human serums. Poultry Sci. 28:622.

Lush, D.: 1943. The chick red cell agglutination test with the virutes of Newcastle disease and fowl plague. Jour. Comp. Path. Therap. 53:157.

Ma, Y. T., Liang, Y., and Tan, P. Y.: 1947. Newcastle disease in Peiping and Shanghai. Chinese Jour. Anim. Husb. Vet. Sci. 8.24. (in Chinese)

McClurkin, A., Sloha, S. K., and Hanson, R. P.: 1954. Rapid diagnosis of Newcastle disease using lung extract. Am. Jour. Vet. Res. 15:314.

McCollum, W. H., and Brandly, C. A.: 1955a. Hemolytic activity of Newcastle disease virus. Am Jour. Vet. Res. 16 584.

McCollum, W. H. and Brandly, C. A.: 1955b. Destruction of virus hemagglutination inhibitor of eggwhite by Newcastle disease virus. Proc. Soc. Exper. Biol. 90:158.

Mack, W. N., and Chotsen, A.: 1955. Beta-propiolactone as a virus altering agent for a Newcastle disease vaccine. Poultry Sci. 34:1010.

Macpherson, L. W.: 1956a. Electron-microscope studies of the virus of Newcastle disease. Canad. Jour. Comp. Med. 20:72.

———. 1956b. Some observations on the epizootiology of Newcastle disease. Canad. Jour. Comp. Med. 20:155.

Maestrone, G., and Coffin, D. C.: 1961. [Study of Newcastle disease by the fluorescent antibody technique.] Arch. Vet. Ital. 12:97, 193. Abstr. in Vet. Bull. 32, no. 162 (1962).

Mansjoer, M.: 1961. Newcastle disease in Indonesia. I. Its present situation, epizootiology and eastern Slovakia combat. Communicationes Veterinariae Sci.

Markham, F. S., Cox, H. R., and Bottorff, C. A.: 1954. Newcastle disease: a serologic study in vaccination and revaccination. Cornell Vet. 44:324.

———, Hammar, A. H., Gingher, P., and Cox, H. R.: 1955. Vaccination against Newcastle disease and infectious bronchitis. I. Preliminary studies in mass vaccination with live virus dust vaccines. Poultry Sci. 34:442.

———, Hammar, A. H., Perry, E. B., and Tesar, W. C.: 1956. Combined Newcastle disease-infectious bronchitis vaccines and the absence of interference phenomena. Cornell Vet. 46:538.

———, Patton, W. H., Hammar, A. H., Bottorff, C. A., Gingher, P. E., Perry, E. D., and Tesar, W. C.: 1957. A second flock history after immunization against Newcastle disease and infectious bronchitis and observations on chronic respiratory disease. Poultry Sci. 36:150.

———, Sylsira, A. W., Hammar, A. H., and Gingher, P.: 1955. A flock history after immunization with a combination Newcastle disease-infectious bronchitis dust vaccine. Poultry Sci. 34:1209.

Matewa, V.: 1960. Vergleichende versuche der Isolierung von Newcastle virus auf Gewebekulturen von Huhnerembryonen und bebrüteten Huhnereieren. Zbl. Bakt. I (Orig.) 178:8.

Meyer, R. F., and Mack, W.: 1946. Personal communication.

Mickalov, J., and Vrtiak, O. J.: 1963. Survival of the virus of Newcastle disease in deep litter. Veterinaristvi 1.9.

Mitchell, G. A., and Walker, R. V. L.: 1951. Note on the infection of a person with Newcastle disease virus. Canad. Jour. Comp. Med. and Vet. Sci. 15:226.

Mitscherlich, E., and Gutjlirk, S.: 1952. Die serologische Untersuchung von Organextrakten gestorbener Hühner auf klassische und atypische Geflügelpest (Newcastle-Krankheit). Deutsch. tierärztl. Wochenschr. 59:571.

Monda, V., Tonga, G., Guarino, C.: 1960. [Antigenic properties of Newcastle disease virus isolated from a sparrow and a canary.] Abstr. in Vet. Bull. 31, no. 3613.

———, Tonga, G., and Guarino, C.: 1961. Isolation and study of the antigenic characteristics of two strains of Newcastle disease virus isolated from the sparrow and the canary. Soc. Ital. delle Scie. Vet. Attil. 14:736.

Monteverde, J. J., Domingo, H. S., Manuel, R. L., Chlalvo, E. J.: 1962. Enfermedades de Newcastle. I. Su hallazo en la Republica Argentina. Ciencas Veterinaria 7:154.

Monti, G.: 1952. La Diagnosi postmortale della pseudopeste aviare mediante la reazione di Hirst. Arch. Vet. Ital. 3:215.

Morehouse, L. G., Moses, H. E., and Gustafson, D. P.: 1963. Newcastle disease virus in tissue culture cells derived from chickens. Am. Jour. Vet. Res. 24:580.

Morimoto, T., Omori, T., Matumoto, M.: 1962. Interference of Russian spring-summer encephalitis virus with Newcastle disease virus in cell culture of bovine embryonic kidney. Jap. Jour. Exper. Med. 32:165.

Moses, H. E.: 1948. Report of the Committee on Newcastle virus properties. Jour. Am. Vet. Med. Assn. 112:126.

———, Brandly, C. A., and Jones, E. E.: 1947. The pH stability of viruses of Newcastle disease and fowl plague. Science 105(2751):477.

Nitzschke, E.: 1954. Zur Frage des Vorkommens von Virusträgern und -ptatauscheidern bei der atypischen Geflügelpest. Berliner Münchener tierärzt. Wochenschr. 67:535.

Olesiuk, O. M.: 1951. Influence of environmental factors on viability of Newcastle disease virus. Am. Jour. Vet. Res. 12:152.

Oppenheimer, F., Milzer, A., Shaughnessy, H., Neal, J., and Levinson, S.: 1944. Production of potent inactivated vaccines with ultraviolet irradiation. Jour. Am. Med. Assn. 125:531.

Osteen, O. L., Mott, L. O., and Gill, E.: 1964. The use of killed virus vaccine to control Newcastle disease. Proc. 64th Ann. Meet. U.S. Livestock Sanit. Assn., p. 232.

Parnell, E. D.: 1950. The keeping quality of shell eggs in storage as affected by Newcastle disease. Poultry Sci. 29:771.

Pereira, H. G., and Gompels, A. E. H.: 1954. The growth of fowl-plague and Newcastle-disease viruses in roller-tube cultures. Jour. Path. Bact. 67:109.

Picken, J. C.: 1964. Thermolability of Newcastle disease virus, p. 167. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

Piraino, F. P., and Hanson, R. P.: 1960. An in vitro method for the identification of strains of Newcastle disease virus. Am. Jour. Vet. Res. 21:125.

Placidi, J.: 1954. En marge de la maladie de Newcastle. Phénomènes nerveux encephalitiques et médullaires (Githagisme) chez les volailles. Rec. Méd. Vét. 130:500.

———: 1956. Accidents consécutifs à la vaccination contre la maladie de Newcastle. Influence de la temperature ambiante. Rôles respectifs du vaccin et l'hôte. Bull. Off. int. Epiz. 45:393.

———, and Santucci, J.: 1956. Agglutination comparée des hématies de la poule, du chameau et des équidés par les virus de la maladie de Newcastle et de la peste aviaire. Ann. Inst. Past. 90:528.

Polci, N., and Silvagni, T.: 1954. Eliminazione del virus di Newcastle da parte di animali carnivori domestici e selvatici contaminati sperimentalmente. Atti Soc. Ital. Sci. Vet. 8.637.

Pomeroy, B. S.: 1948. Personal communication.

———: 1951. Newcastle disease of poultry. Prog. Rep. to Tech. Committee of North Central Region. Nov. 14, 1951, p. 25.

———, and Brandly, C. A.: 1953. Facts about Newcastle disease. Agr. Exper. Sta. Univ. of Minn. North Central Regional Publication No. 34, 22 pages.

———, and Fenstermacher, R.: 1948. Newcastle disease is spreading. U.S. Egg Poultry Mag. 54:19.

Price, R. J., Bottorff, C. A., Seeger, K., Sylstra, A. W., and Markham, F. S.: 1955. Vaccination against Newcastle disease and infectious bronchitis. 2 Field trials in mass vaccination with live virus dust vaccines. Poultry Sci. 34:449.

Prier, J. E., and Alberts, J. O.: 1950. Studies on Newcastle disease. VII. Viability of live embryo Newcastle disease virus in buffered glycol. Cornell Vet. 40.300.

———, Millen, T. W., and Alberts, J. O.: 1950. Studies on Newcastle disease. IV. The presence of NDV in eggs of hens vaccinated with live vaccine. Jour. Am. Vet. Med. Assn. 116.54.

Quinn, R. W., Hanson, R. P., Brown, J. W., and Brandly, C. A.: 1952. Newcastle disease virus in man. Clinical and virus isolation studies in 3 cases. Jour. Lab. Clin. Med. 40:736.

Raggi, L. G.: 1960. A rapid macroscopic plate agglutination test for Newcastle disease—a preliminary report. Avian Dis. 4:320.

———, Lee, G. G., and Schrab-Haghighat, V.: 1963. Infectious bronchitis virus interference with growth of Newcastle disease virus. I. Study of interference in chicken embryos. Avian Dis. 7:106.

Reagan, R. L., and Brueckner, A. L.: 1951. Response of the cave bat (Myotus lucifugus) to Newcastle disease virus by various methods of exposure. Cornell Vet. 41:56.

———, Lillie, M. G., Hauser, J. E., Poelma, L. J., and Brueckner, A. L.: 1947. Response of monkeys to poliomyelitis after injection with Newcastle disease virus. Proc. 51st Ann. Meet. U.S. Livestock Sanit. Assn., p. 55.

Reid, J.: 1955. Fowl pest. Agriculture, London 61:465.

Reid, John: 1961. The control of Newcastle disease in Great Britain. Br. Vet. Jour. 117:275.

Report of Chief of Bureau of Animal Industry: 1948. P. 43.

———: 1950. Pp. 47, 87.

Report of the Committee on Fowl Pest Policy: 1962. Her Majesty's Stationery Office, London, Command 1664.

Report of Director: 1950. Purdue University, 63rd Ann. Rep. Univ. Agr. Exper. Sta. for year ending June 30, 1950. P. 55.

Report of Poultry Disease Subcommittee on Animal Health, Agr. Bd., Div. of Biol. and Agr., Nat. Acad. of Sciences, Nat. Res Council, Washington, D.C.: 1959. Methods for the Examination of Poultry Biologics, 1st ed. Publication 705.

———: 1963. Methods for the Examination of Poultry Biologics, 2nd ed. (revised). Publication 1038.

Report of the Subcommittee on Plans for Eradication of Newcastle Disease: 1959. North Central Regional Technical Committee, NC-6, Nov. 28–29, 1959, Chicago, Illinois.

Richter, J. H. M.: 1956. Een proefondervindelijk onderzoek naar kippenpokken en pseudo-vogelpest. Tijdschr. v. Diergeneesk. 81:763.

Rott, R.: 1964. Antigenicity of Newcastle disease virus, p. 133. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press. Madison.

———, and Schäfer, W.: 1961. Fine structure of subunits isolated from Newcastle disease virus. Virology 14:298.

———, and Schäfer, W.: 1962. Hydroxylamin-Empfindlichkeit des Newcastle Disease Virus (NDV). Z. Naturforsch. 17b.861.

Rousseff, G., and Mateff, G.: 1956. Préparation d'un vaccin contre la maladie de Newcastle avec la souche "F" et vérification de son innocuité. Bull. Off. int. Epiz. 45:409.

Rubin, H., and Franklin, R. M.: 1957. On the mechanism of Newcastle disease virus neutralization by immune serum. Virology 3.84.

———, Franklin, R. M., and Baluda, M.: 1957. Infection and growth of Newcastle disease virus (NDV) in cultures of chick embryo lung epithelium. Virology 3.587.

Russell, C. G.: 1956. Mengenabhängigkeitsverhältnis zwischen interferierenden Virusstämmen der atypischen Geflügelpest bei Versuchen an Hühnern. Arch. exper. VetMed. 10.207.

Sagik, B. P., and Levine, S.: 1957. The interaction of Newcastle disease virus (NDV) with chicken erythrocytes: attachment, elution, and hemolysis. Virology S.401.

Sahai, L.: 1937. Doyle's disease of fowls: Its diagnosis and control. Agr. Livestock India 7:11.

Salk, J. E.: 1944. A simplified procedure for titrating hemagglutinating capacity of influenza virus and the corresponding antibody. Jour. Immunol. 49:87.

Santoni, A., and Bonaduce, A.: 1952. Sul potere patogeno del virus della pseudopeste dei polli per l'occhio e sulla possibilità di migrazione del virus da un occhio all'altro. Bul. ocul., Toulouse 31:129.

Schäfer, W.: 1963. Structure of some animal viruses and significance of their components. Bacteriol. Rev. 27(1):1.

——— and Rott, R.: 1959. Untersuchungen des Newcastle Disease und Mumps-Virus. Z. Naturforsch. 14b.629.

Schloer, Gertrude: 1961. Plaque characteristics of Newcastle disease virus. Univ. of Wis. doctoral thesis.

Schmidl, U., and Bindrich, H.: 1956. Zur Frage der Ausscheidung und Vermehrung des Virus der atypischen Geflugelpest nach Infektion immuner Huhner. Arch. Exper. VetMed. 10 649.

Schoening, H. W., and Osteen, O. L.: 1918. Newcastle disease in the United States of America. Proc. Eighth World's Poultry Cong. I:636.

Schoop, G., Seigert, R., Galassi, D., and Kloppel, G.: 1955. Newcastle-Infektionen beim Steinkauz (Athene noctua), Hornraben (Bucorvus sp.) Seeadler Haliaetus albicilla) und Riesenalkvogel (Dacelo gigas). Monatsh. Tierheilk. 7:223.

Scott, G. R., Gibson, M. A., and Dansken, D.: 1956. The reappearance of Newcastle disease in Kenya. Bul. Epiz. Dis. Afr. 4.65.

——— Hanson, R. P., and Brandly, C. A.: 1955. A simple procedure for the in vitro propagation of Newcastle disease virus. Proc. 90th Ann. Meet. Vet. Med. Assn., p. 312.

Seiffert, G.: 1955 Zuchtung von Geflugelpest und anderen Viren nach einer neuen Methode. Berliner Munchener tierarztl. Wochenschr. 68.388.

Shah, K. V., and Johnson, H. N.: 1959. Isolation of Ranikhet (Newcastle) virus from a fledgeling koel (Eudynamys scolopaceus) by intracerebral inoculation in mice. Indian Jour. Med. Res. 47:604.

Shelikov, A., Vogel, J. E., and Chi, L.: 1958. Hemadsorption (adsorption-hemagglutination) test for viral agents in tissue culture with special reference to influenza. Proc. Soc. Exper. Biol. and Med. 97:802.

Shimizu, T., Kumagai, T., Ikeda, S., and Matumoto, M.: 1961. A new in vitro method (END) for detection and measurement of hog cholera virus and its antibody by means of effect of HC virus on Newcastle disease virus in swine tissue culture. III. END neutralization test. Arch. ges Virusforsch. 11.215.

Shimkin, N. J.: 1946. Conjunctival haemorrhage due to an infection of Newcastle virus of fowls in man. Brit. Jour. Ophthal. 30:260.

Silverstein, Samuel C., and Marcus, Philip I.: 1964. Early stages of Newcastle disease virus-Hela cell interaction, an electron microscopic study Virology 23.370.

Sinha, S. K.: 1957. Studies on experimental epizootiology of Newcastle disease virus in chickens. Univ. of Wis doctoral thesis

——— 1958. Influence of temperature of incubation of embryonating eggs following inoculation of Newcastle disease virus Avian Dis 2:138.

——— Hanson, R. P., and Brandly, C. A.: 1952. Comparison of the tropisms of six strains of Newcastle disease virus in chickens following aerosol infection. Jour. Infect. Dis. 91:276.

——— Hanson, R. P., and Brandly, C. A.: 1954. Aerosol transmission of Newcastle disease in chickens. Am. Jour. Vet. Res. 15:287.

——— Hanson, R. P., and Brandly, C. A.: 1957. Effect of environmental temperature upon facility of aerosol transmission of infection and severity of Newcastle disease among chickens. Jour. Infect. Dis. 100:162.

Spalatin, H. J., Lulié, V., and Greguné, J.: 1953. Mukteswar vakcina atipicne kuge peradi (Proizvodnja I Primjena). Vet. Arhiv. 23:253.

Takamatsu, Y., Miyamoto, T., Zairen, K., Kawakubo, A., and Nagashima, M.: 1956. Outbreaks of Newcastle disease in Tokyo in the spring of 1951. NIBS Bul. Biol. Res. 1.63.

Technical Committee on Newcastle Disease of the North Central Region, Report of the Cooperating Stations. Meet. of Nov. 1951 at Chicago, Ill. Mimeo.

Technical Committee on Newcastle Disease of the North Central Region. Report of the Subcommittee on Vaccine Evaluation. (Hanson and Beamer) Meet. of Nov. 1951, at Chicago, Ill. Mimeo.

Thiry, L.: 1963. Chemical mutagenesis of Newcastle disease virus. Virology 19.225.

Thompson, C. H. Jr., and Osteen, O. L.: 1948. A technique for the isolation of Newcastle disease virus using streptomycin as a bacterial inhibitor. Ann. Jour. Vet. Res. 9.303.

——— and Osteen, O. L.: 1952. Immunological and pathological findings on a highly virulent strain of Newcastle disease virus from Mexico. Am. Jour. Vet. Res. 13:407.

Tilley, F. N., and Anderson, W. A.: 1947. Germicidal action of certain chemicals on the virus of Newcastle disease. Vet. Med. 42:229.

Tolba, M. K., and Eskarous, J. K.: 1962. Effect of temperature on the hemagglutination activities and infectivity to chick embryos of different strains of Newcastle disease and fowl plague viruses. Arch. f. Kreislaufforsch. 38 234.

Topacio, T.: 1934. Cultivation of avian pest virus (Newcastle disease) in tissue culture. Philippine Jour. Sci. 53:245 and Philippine Jour. Anim. Ind. 4 50.

Traub, E.: 1943–44. Weitere Mitteilungen über die Aktiv-Immunisierung mit Absorbat-Impfstoffen gegen die atypische Geflugelpest. Zeitschr. infekt. Krankh. Haustiere 60.567.

Upton, E., Hanson, R. P., and Brandly, C. A.: 1953. Antigenic differences among strains of Newcastle disease virus. Proc. Soc. Exper. Biol. and Med. 84:691.

———, Hanson, R. P., Dow, D., and Brandly, C. A.: 1953. Studies on intracerebral inoculation of Newcastle disease virus (NDV) into mice. 1. Response of weanling mice to 25 strains of NDV. Jour. Infect. Dis. 92:175.

———, Hanson, R. P., Tepley, N. W., and Brandly, C. A.: 1951. Neurotoxicity of Newcastle disease virus (NDV) for hamsters and mice. Proc. 51st Ann. Meet. Soc. Am. Bact., p. 87.

Valadao, F. G.: 1955. The H.I. test for the diagnosis of Newcastle disease in Mozambique. Its value as an indication of immunity. Bul. Epiz. Dis. Afr. 3.373.

Valentine, R. C., and Isaacs, A.: 1957. The structure of viruses of the Newcastle disease-mumps-influenza (Myxovirus) group. Jour. Gen. Microbiol. 16 680.

Van Roekel, H.: 1946. Proc. Conf. Newcastle disease, U.S. Dept. Agr., p. 17.

———: 1955. An evaluation of Newcastle disease-wing-web vaccine. Proc. 92nd Ann. Meet. Am. Vet. Med. Assn., p. 324.

———, Sperling, F. G., Bullis, K. L., and Olesiuk, O. M.: 1948. Immunization of chickens against Newcastle disease. Jour. Am. Vet. Med. Assn. 112:131.

Van Waveren, G. M., and Zuijdam, D. M.: 1955. Vaccinatie tegen pseudovogelpest (Newcastle disease) door het mengen van entstof door het drinkwater. Tijdschr. v. Diergeneesk. 80 685.

Vrtiak, J.: 1958. [Epidemiology of Newcastle disease in eastern Slovakia.] Abst. from Vet. Bul. (1959) 29.312.

Walker, R. V. L.: 1948. Newcastle disease. Canad. Jour. Comp. Med. 12:171.

———: 1952. Studies in Newcastle disease. IV. Rapid methods of diagnosis. Canad. Jour. Comp. Med. 16.333.

———, Gwatkin, R., and McKercher, P. D.: 1953. Efficiency of a quaternary ammonium glycol mixture against Newcastle disease virus and Salmonella pullorum. Canad. Jour. Comp. Med. 17.223

———, and McKercher, P. D.: 1954. Studies in Newcastle disease. IX. Further investigation of the carrier problem. Canad. Jour. Comp. Med. 18:431.

———, McKercher, P. D., and Bannister, G. L.: 1954. Studies in Newcastle disease. VII. The possible role of the pigeon as a carrier. Canad. Jour. Comp. Med. 18:244.

———, and Powell, E. P. B.: 1950. Newcastle disease, a study of the carrier problem. Canad. Jour. Comp. Med. 14:61.

Waterson, A. P.: 1964. The morphology and compositions of Newcastle disease virus, p. 119. In R. P. Hanson (ed.), Newcastle Disease Virus: An Evolving Pathogen. University of Wisconsin Press, Madison.

———, and Cruickshank, J. G.: 1963. The effect of ether on Newcastle disease virus. A morphological study of eight strains. Z. Naturforsch. 18b:114.

Wenner, H. A., and Lash, B.: 1949. Chorio-meningo encephalitis following inoculation of Newcastle disease virus in rhesus monkeys. Proc. Soc. Exper. Biol. and Med. 70.263.

Wheelock, E. F.: 1963. Intracellular site of Newcastle disease virus nucleic acid synthesis. Proc. Soc. for Exper. Biol. and Med. 114.56.

Williamson, A. P., Blattner, R. J., and Simonsen, L.: 1956. Mechanism of the teratogenic action of Newcastle disease virus on the chicken embryo. Jour. Immunol. 76.215.

Wilson, J. E.: 1952. Newcastle disease in a Gannet. Sole Jeanne. A preliminary note. Vet. Record 62:33.

Winslow, N. S., Hanson, R. P., Upton, E., and Brandly, C. A.: 1950a. Effect of homologous antiserum on Newcastle disease virus infection of embryonating eggs. Proc. Soc. Am. Bact., p. 73.

———, Hanson, R. P., Upton, E., and Brandly, C. A.: 1950b. Agglutination of mammalian erythrocytes by Newcastle disease virus. Proc. Soc. Exper. Biol. 74.171.

Winterfield, R. W., Goldman, C. L., and Seadale, E. H.: 1957. Newcastle disease immunization studies. 4. Vaccination of chickens with B_1, F and LaSota strains of Newcastle disease virus administered through the drinking water. Poultry Sci. 36:1076.

———, and Seadale, E. H.: 1956. Newcastle disease immunization studies. 1. Viability of Newcastle disease virus administered as a vaccine in the drinking water. Am. Jour. Vet. Res. 17.5

———, and Seadale, E. H.: 1957a. Newcastle disease immunization studies. 2. The immune response of chickens vaccinated with B_1 Newcastle disease virus administered through the drinking water. Poultry Sci. 36 54

———, and Seadale, E. H.: 1957b. Newcastle disease immunization studies. 3. The immune response of chickens vaccinated at an early age with B_1 Newcastle disease virus administered through the drinking water under field conditions. Poultry Sci. 36 65.

Woernle, H., and Brunner, A.: 1957. Zur Übertragungsmöglichkeit der atypischen Geflügelpest (Newcastle Disease) durch schutzgeimpfte und später infizierte Hühner. Monatsh. Tierheilk. 9:116.

Wolfe, H. R., and Dilks, E.: 1948. Precipitin production in chickens. III. The variation in the antibody response as correlated with the age of the animal. Jour. Immunol. 58:245.

Yates, V. J., and Fry, D. E.: 1957. Observations on a chicken embryo lethal orphan (CELO) virus. Am. Jour Vet. Res. 18.657.

Zakomirdin, A. A.: 1963. Biothermal disinfection of manure from poultry with Newcastle disease and infectious laryngotracheitis. Veterinariya, Moscow 40(9):64.

Zargar, S. L., and Pomeroy, B. S.: 1949. A rapid whole blood plate test for the diagnosis of Newcastle disease. Jour. Am. Vet. Med. Assn. 115:554.

———, and Pomeroy, B. S: 1950. Isolation of Newcastle disease from commercial fowlpox and laryngotracheitis vaccines. Jour. Am. Vet. Med. Assn. 116.504.

Zuijdam, D. M.: 1949. Pseudovogelpest (Newcastle disease) bij Nederland geïmporteer Fazanten. Tijdschr v. Diergeneesk. 74:481.

———: 1951. Onderzoek naar de verspreiding van pseudo-vogelpestvirus door wilde ratten. Tijdschr. v. Diergeneesk. 76:237.

———: 1952a. Isolation of Newcastle disease virus from the osprey and the parakeet. Jour. Am. Vet. Med. Assn. 120.88.

———: 1952b. Pseudovogelpest vaccinatie en virusuitscheiding. N. V. Drukkerij P. Den Boer, Utrecht. (English summary.) P. 123.

———: 1953. Vaccination against Newcastle disease. Proc. 15th Internat. Vet. Cong. Stockholm 1:252.

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23

Ornithosis

In his thesis written in 1895, "De la psit-tacose ou infection spéciale déterminée par des perruches," Antonin Morange intro-duced the term "psittacosis" for a clinically defined disease of man arising from associ-ation with parrots, and, because of its ap-propriateness to what was then known of the infection, this term found wide usage. Much later, as it was learned that a great variety of birds, including poultry, can be affected, the more descriptive, more general term "ornithosis" was introduced (Meyer, 1942). Psittacosis is the term used for a generalized infection man contracts from birds and for the infections caused by the same agent in psittacine birds. Ornithosis, regarded as an equivalent term in the sixth revision of the *International List of Dis-eases and Causes of Death* and as a syno-nym in the eighth edition of *Control of Communicable Diseases of Man* (Official Report Am. Pub. Health Assn., 1955), is re-served for the infection in extrapsittacine birds. The known history of the infection

clarifies how the term psittacosis estab-lished itself. The imprint of the pandemic of the early 1930's, before the advent of the antimicrobial drugs, has not faded. But preoccupation with the psittacine aspects too long limited the pursuit of the infective agent, not only in wild and domesticated birds, but also in mammals, where its parasitism is being increasingly recognized.

Acute ornithosis is at times a severe dis-ease of pigeons, ducks, and turkeys. This true zoo-anthroponosis has been the source of single or extensive group infections throughout all occupational groups that come into contact with infected fowl. The host range is wide and cosmopolitan, and the steadily increasing number and kinds of avian hosts have altered the concepts on the infection chains. The importance of sources of human psittacosis other than cage pets became more apparent than ever during the 1950's. At the same time, growth of the pet bird trade after World War II increased the incidence of human

[675]

psittacosis from that source. As an occupational disease among pigeon breeders, poultry farmers, and poultry processing plant employees, it has created new problems. Fortunately the mortality rate has been lowered by use of the wide-spectrum antimicrobial drugs.

HISTORY

The little-known history of ornithosis in poultry has been recorded only since 1930, and the few existing fragments described, mainly by workers interested not in poultry but in the organism or the infection in man, must be pieced together. The result cannot be considered a true picture of the progress of the disease in either poultry or man.

By the time of the 1929 and 1930 epidemics of psittacosis due to contact with cage birds, methods of studying microorganisms had been improved, and as experimental hosts were sought and means of transmission and sources of infection were studied, some investigators (Bedson and Western, 1930; Dahmen and Hamet, 1930; Krumwiede et al., 1930; Levinthal, 1930; Meyer, 1935) used chickens in their experimental work on the psittacosis agent. In one experiment, Bedson and Western (1930) attempted infection of pigeons but failed.

That pigeons are infectable was first learned through a natural infection in Johannesburg in March, 1939, when a pigeon fancier sent 2 birds to the Onderstepoort Laboratories, Department of Agriculture, Union of South Africa, because he was losing a few birds from his flock of 200 to 300 (Coles, 1940). Naturally acquired infections were soon reported in racing and carrier pigeons in California, and there was good circumstantial evidence of 2 human infections acquired from a sick feral pigeon in New York City (Meyer, 1941; Meyer et al., 1942a). The bulk of subsequent reports on human psittacosis arising from contact with pigeons has been concerned with racing and fancy pigeons rather than poultry pigeons, but there is serologic evidence that squab raisers do contract it and that it is an infection of poultry pigeons in the United States. The first known infection was contracted through contact with racing pigeons in California (Meyer et al., 1942a). The isolate from the lung of the patient was indistinguishable from that from the organs of pigeons to which the victim was exposed for only a short time.

Inevitably a natural infection in poultry was identified, in 1939, after serodiagnosis of psittacosis was made in a case of atypical pneumonia in New Jersey (Meyer and Eddie, 1942). The patient had tended a flock of 700 White Leghorn chickens and had dressed birds from the flock. The serum of 3 other members of the family also reacted in the complement fixation test. The agent was isolated from 1 of 3 chickens tested at the Hooper Foundation—the first isolation from a naturally infected fowl. This may have been an instance of epizootic ornithosis, but the death of 500 birds from "range paralysis" and of over 100 additional birds, an unusually high mortality for ornithosis in poultry, especially chickens, makes it seem probable that some other infection was also active in the flock at the time.

Serologic evidence of naturally acquired ornithosis in ducks and turkeys was provided by a survey of these fowl in Michigan in 1942 (Eddie and Francis, 1942). Within 3 years human infections due to contact with ducks were found in California (Meyer and Eddie, 1952) and in New York (Wolins, 1948; Korns, 1955). The Long Island outbreak is interesting in several respects. It is the first poultry outbreak in which an extensive epidemiologic and serologic investigation was made. These revealed a higher incidence of clinical and subclinical infections in duck handlers, hence the first proof of the occupational importance of the disease in poultry workers. It showed that the situation with respect to poultry is similar in some ways to that of cage birds—that infection of man may result from only brief contact and that many subclinical infections take place. Isolates from wild birds (feral pigeons and sea

gulls) were also found in the course of the study. The agent was isolated from sick and well ducks, and the incidence of positive seroreactors was high (40.2 per cent). The disease in the ducks was not of primary concern here, and reports do not describe this in any detail. There were sick ducks and there were concurrent *Salmonella typhimurium*, *Pasturella multocida*, *Pfeifferella anatipestifer*, and other infections.

Extensive research initiated in 1952 resulted in isolation of *Bedsoniae* from patients, ducks, geese, and sea gulls, and learning that young ducks were a main source of infection in Bohemia, Moravia, and Silesia. The highest incidence in man was in the age group of 40 to 60 years. The infection was not always present, but fatal cases and reinfections were observed (Strauss, 1957). Uncontrolled transfer of poultry between Bohemia and Slovakia or of ducks from Hungary to East Slovakia resulted in epizootics on duck farms. There were serious economic losses (an estimated mortality of 10,000 duck embryos and 30,000 hatched ducklings), clinical psittacosis of 10 employees and serologic evidence of infection in 24 of 213 workers on other farms. Annual ornithosis epizootics have become a serious health problem (Strauss *et al.*, 1960). Ornithosis in ducks followed by human causes has been reported from Austria (Fürst *et al.*, 1957), Poland (Parnas *et al.*, 1960) and Roumania (Sarateanu *et al.*, 1960).

In 1958 German medical and later veterinary publications began to call attention to the increasing number of ornithosis outbreaks in poultry processing plants and in breeding and fattening farms in the German Democratic Republic. Between 1958 and 1960, 783 human infections were attributed to contact with ducks, chickens, geese, pigeons, or poultry in general (Ortel, 1960, 1961, 1963). The clinical disease described in several excellent articles (Voight *et al.*, 1962; Siegmund, 1960; Reinwein and Walther, 1961; Kukowka, 1961c; Otto, 1962; Gneuss and Koitzsch, 1961) can be conclusively diagnosed as ornithosis only

by isolation or serologic tests. In the outbreak in Halle, sputum and throat washings yielded a bedsonia in 10 patients (Ortel, 1960). One third of 71 sick or dead ducks and ducklings from a breeding and fattening farm in northeast Germany harbored bedsonia (Illner, 1962b). In serologic screening of 70 flocks, 1,428 ducks were examined in the direct complement fixation test: 233 from 35 flocks were considered positive, 5 flocks suspicious, and 30 uninfected (Lehnert and Hille, 1960).

The first report that ornithosis can infect the turkey was fairly recent (Irons *et al.*, 1951, 1955), and in subsequent outbreaks many isolates from turkeys and from human beings have been of unusual virulence for the indicator host, the mouse. In several outbreaks the mortality rate in the flocks has been much higher than that formerly noticed in poultry (Osgood *et al.*, 1956; Francis, 1960). On the other hand, in other flocks without unusual illness but with gross fesions of ornithosis, as high as 13.8 per cent have been condemned by inspectors at the time of slaughter and have caused human outbreaks (Rich *et al.*, 1962).

An important observation was made in 1954 in California (Meyer and Eddie, 1956b; Meyer, 1959b; Page, 1959c, 1960): *bedsoniae of low pathogenicity for mice and guinea pigs produced gross visceral lesions, but rarely illness or deaths in turkeys.* Isolates of similar low pathogenicity have been encountered in tissues of other flocks in California (Page, 1960), in Michigan (Meyer, 1959b), in Minnesota (Pomeroy *et al.*, 1957; Graber and Pomeroy, 1958; Graber, 1959; Gale, 1960) and recently in Oregon on a ranch where a highly virulent bedsonia had caused severe ornithosis since 1956 (Meyer and Eddie, 1962b). The first California observation was of particular interest to the epidemiologist: 88 employees of a poultry plant were exposed during processing of 2 different batches of turkeys at two different times; 37 to 83 per cent of the turkeys were seropositive. *None became ill and only 3 showed serologic evidence of experience with bedsonia, even though during one processing 900 pounds*

of visibly diseased viscera were condemned.

Originally it was reasoned that the low pathogenicity of the isolate from the epizootic was responsible for the absence of human infection, but the large reservoir of ornithosis in domestic turkeys in Wisconsin and Minnesota, proved by serologic tests to exceed 40 per cent of the tested flocks, caused clinical and subclinical human ornithosis of sporadic nature in 1956 to 1960 (Graber and Pomeroy, 1958; Graber, 1959). From 16 to 29 per cent of the over 200 poultry workers examined reacted in the complement fixation test in dilutions of 1:8 and above. According to serologic screening surveys, bedsonia isolates of low pathogenicity for mice are widely spread in the turkey population of the United States. Recently ornithosis in one turkey has been reported from Great Britain (Grattan, 1963). They are quite similar to the isolates from pigeons, ducks, and even some psittacine birds. How and why they become highly toxic and pathogenic for mammals including man cannot be answered because the history of ornithosis as a disease of poultry is largely unknown.

Major changes in poultry-raising practices in this country could change the importance of infectious diseases of poultry in general. Individual owners of small flocks have probably accepted minor losses, some in all probability due to mild epizootics of ornithosis, but when poultry birds are raised in large groups, under some circumstances affording much better opportunities for intraflock spread, the economics of the infection also changes. The new outbreaks in the costly turkey, with a mortality rate that can no longer be ignored, a higher concentration of the cost of the infection to a single flock owner, and the occurrence of clinical infections in heavily exposed processing plant workers warn that the position of this infection may be undergoing a change. Recently the turkey in the United States and the duck in eastern Europe have come into as much prominence as a source of infection in man as cage pets had in former years.

Infections have been found in domestic pheasants (Ward and Birge, 1952; Meyer and Eddie, 1956b) and geese (Strauss, 1956; Fürst et al., 1957), but not yet in the guinea fowl. Among wild birds used for food, the fulmar (Haagen and Mauer, 1938) and the muttonbird (Mykytowycz et al., 1955) bear mention. There are no known instances of transmission of the virus through ingestion of poultry.

There have been single cases and outbreaks in human beings (5,390 cases, 89 deaths) due to poultry and other food birds (Table 23.1). There are several reports in which ornithosis is considered primarily as a poultry disease, in pigeons (Hughes, 1947) and in turkeys (Pate et al., 1954; Davis, 1955; Davis and Delaplane, 1955; Davis et al., 1957a, b; Pomeroy et al., 1957), but its history as a disease of poultry is largely unknown.

Occasionally an astute physician encounters a case of pneumonia in which the clinical picture deviates enough from the pneumococcal pneumonias to arouse his suspicions so that he must look for the cause elsewhere. The patient's history leads to poultry. In most cases the patient recovers and no further inquiry is made. Under exceptional circumstances the matter has been pursued further, and something is learned about the infection in the responsible flock, but mainly in relation to the human infection, not as a subject in itself. Almost never is the epidemiologic investigation carried beyond identification of the source of a human infection. Some groups particularly interested in ornithosis have made small surveys in a limited area usually in only one season when there have been human infections, and they have found the bedsonia or serologic evidence in poultry flocks. Published reports again and again describe evidence of the infection in poultry and in people who come into contact with poultry at any point on its way to market, but this information usually comes after the flock has already been marketed and all useful material for study has been destroyed. It is obviously then already too late for a careful study of the course of the

disease in the flock. Since the people now concerned in preparation of poultry for the market constitute an occupational group, the infection is becoming the subject of wider interest again, and again it is the spread to man that is the stimulus.

Its position as a poultry disease has simply never been defined, probably at least partly because the mortality rate in the flocks, as far as is known, has usually been relatively low. Since writers on this subject have been mainly physicians, epidemiologists and public health workers, and only recently veterinarians, the interests of these groups are reflected, and its course in the responsible poultry is almost never dealt with at all beyond mention of sick birds.

In the 1959 edition of this book, based on statistics for the period 1931 to 1956 maintained by the George Williams Hooper Foundation and published reports, the incidence of human psittacosis in 19 countries attributed to contact with pigeons, turkeys, ducks, chickens, and seashore birds had been tabulated. Bringing these data up to 1963, the following conclusions are suggested: (a) With the exception of the United States, sporadic cases attributed to exposure to pigeons are rarely reported or described. (b) The records rarely make a sharp distinction between clinical or merely serologically recognized cases. Therefore, the figures include all the reported cases. (c) Statistics for 9 additional countries have been added. (d) The marked increase in occupational infection in the duck raising and processing industries of Czechoslovakia, East Germany, and Hungary and in the turkey industry of North America due to epizootics of ornithosis in the poultry flock has increased the total number of human psittacosis cases attributable to poultry to 5,390 with 89 deaths (Table 23.1). The absence of statistics cannot be accepted as proof that human psittacosis does not exist in countries not included in the tabulation. What is more important, however, is the likely inadequacy of the tabulation for other reasons: In view of the wide host range of the many species

of bedsoniae of proven infectivity for mammals in the vicinity of man, it is reasonable to suspect that infections must occur more frequently, but they are not recognized except under special circumstances.

Search for the cause of atypical pneumonia in the U.S.A. and Europe indicated that in from 5 to 16 per cent the serologic evidence was in keeping with a diagnosis of psittacosis (Smadel, 1943; Eaton, 1945; Westwood, 1953; Lepine and Sautter, 1951; Jansson, 1960, and others). It is not known precisely how many cases of the pneumonitis originated from feral pigeons. According to large serological surveys to measure the incidence and distribution of psittacosis within (a) social, urban, and rural groups and (b) certain occupational groups, more people had been infected with bedsoniae than is indicated in Table 23.1. The reaction rate of one group of poultry plant workers in Connecticut exposed for several years was 24 per cent (Rindge et al., 1959); it reached 68 to 75 per cent in poultry processors in Texas and Oregon (Osgood et al., 1956). Valuable data collected in Czechoslovakia by Serý and his associates (1961) and Strauss and Serý (1964) are even more informative since only complement fixation titers higher than 1:32 on one serum specimen were considered indicative of past or present psittacosis. The average percentage of reactors in healthy persons living in rural areas was 15 per cent, but in 213 employees on poultry farms it rose to 22.5 per cent, and to 74 per cent in those who had worked from 3 months to 5 years in the contaminated environment. It may be mere coincidence but 52 per cent of 63 keepers of pigeons in Czechoslovakia corresponds with the 35 per cent of 169 pigeon breeders in Germany (Mohr, 1954; Siegmund, 1960; Wohlrab, 1955). Similar findings of high (39-50 per cent) incidences in occupational groups have been reported from Germany and Roumania (Sarateanu et al., 1961). Regional as yet unexplained differences, e.g., only 5 per cent reactors in 1,527 poultry plant workers in Poland reacted in dilutions of 1:8 and above, have been pub-

of visibly diseased viscera were condemned.

Originally it was reasoned that the low pathogenicity of the isolate from the epizootic was responsible for the absence of human infection, but the large reservoir of ornithosis in domestic turkeys in Wisconsin and Minnesota, proved by serofogic tests to exceed 40 per cent of the tested flocks, caused clinical and subclinical human ornithosis of sporadic nature in 1956 to 1960 (Graber and Pomeroy, 1958; Graber, 1959). From 16 to 29 per cent of the over 200 poultry workers examined reacted in the complement fixation test in dilutions of 1:8 and above. According to serologic screening surveys, bedsonia isolates of low pathogenicity for mice are widely spread in the turkey population of the United States. Recently ornithosis in one turkey has been reported from Great Britain (Grattan, 1963). They are quite similar to the isolates from pigeons, ducks, and even some psittacine birds. How and why they become highly toxic and pathogenic for mammals including man cannot be answered because the history of ornithosis as a disease of poultry is largely unknown.

Major changes in poultry-raising practices in this country could change the importance of infectious diseases of poultry in general. Individual owners of small flocks have probably accepted minor losses, some in all probability due to mild epizootics of ornithosis, but when poultry birds are raised in large groups, under some circumstances affording much better opportunities for intraflock spread, the economics of the infection also changes. The new outbreaks in the costly turkey, with a mortality rate that can no longer be ignored, a higher concentration of the cost of the infection to a single flock owner, and the occurrence of clinical infections in heavily exposed processing plant workers warn that the position of this infection may be undergoing a change. Recently the turkey in the United States and the duck in eastern Europe have come into as much prominence as a source of infection in man as cage pets had in former years.

Infections have been found in domestic pheasants (Ward and Birge, 1952; Meyer and Eddie, 1956b) and geese (Strauss, 1956; Fürst et al., 1957), but not yet in the guinea fowl. Among wild birds used for food, the fulmar (Haagen and Mauer, 1938) and the muttonbird (Mykytowycz et al., 1955) bear mention. There are no known instances of transmission of the virus through ingestion of poultry.

There have been single cases and outbreaks in human beings (5,390 cases, 89 deaths) due to poultry and other food birds (Table 23.1). There are several reports in which ornithosis is considered primarily as a poultry disease, in pigeons (Hughes, 1947) and in turkeys (Pate et al., 1954; Davis, 1955; Davis and Delaplane, 1955; Davis et al., 1957a, b; Pomeroy et al., 1957), but its history as a disease of poultry is largely unknown.

Occasionally an astute physician encounters a case of pneumonia in which the clinical picture deviates enough from the pneumococcal pneumonias to arouse his suspicions so that he must look for the cause elsewhere. The patient's history leads to poultry. In most cases the patient recovers and no further inquiry is made. Under exceptional circumstances the matter has been pursued further, and something is learned about the infection in the responsible flock, but mainly in relation to the human infection, not as a subject in itself. Almost never is the epidemiologic investigation carried beyond identification of the source of a human infection. Some groups particularly interested in ornithosis have made small surveys in a limited area usually in only one season when there have been human infections, and they have found the bedsonia or serologic evidence in poultry flocks. Published reports again and again describe evidence of the infection in poultry and in people who come into contact with poultry at any point on its way to market, but this information usually comes after the flock has already been marketed and all useful material for study has been destroyed. It is obviously then already too late for a careful study of the course of the

TABLE 23.1 (continued)

Reported Incomplete Incidences by Countries of Human Psittacosis From 1931–1963 Contact With Food-Producing Birds

Country	Pigeons C*	Pigeons D†	Ducks C*	Ducks D†	Turkeys C*	Turkeys D†	Chicken C*	Chicken D†	"Poultry" C*	"Poultry" D†	Seashore Birds C*	Seashore Birds D†	Total C*	Total D†	Source of Information
Iceland											5	2	5	2	Bedson, personal communication
India	1			6									9	6	Kalra, 1958
Israel	24												24		Berman et al., 1955; Komarov and Goldsmit, 1952
Italy	70(?)												71		Olivo and Badiali 1956; Lippi et al., 1960
Japan	2 at least												2		Matumoto et al., 1960
Netherlands	(154) 1951-54 205 (1951-61)												(154) 205	(2)	Jansen, 1955; Dekking, 1963; Miles, 1959a
New Zealand	+(2)												(17)		Schmidtke, 1957
Norway	(17)		9		(2, 4 farms)				(54)				9(54)	2	Sarateanu et al., 1960, Busila et al., 1960; Popovici and May, 1960
Roumania								(4)					(8)		Grubb, 1955
Sweden	(4)												(6)		Meyer and Genewein, 1957
Switzerland	5						1								
USA	164		35	8	656	12	64		12 4 pheasants 2 grouse		1		931 4	21	Hooper Foundation and Communicable Disease Center Record
USSR	+		+Endemic						304				304	2	Terskikh, 1954 and 1964, Bezdenezhnykh, 1960, Kortev and Fedorova, 1963
Yugoslavia	6			1?									7	1?	Teran, 1958
Totals	680 (177)	19	1251(30)	16/1*	754	12	285(6)	12	2226(54)	2	192	–	5390(265) 40	169/1*	

[681]

* C.=Cases   † D=Deaths   ( ) Data based on personal information.

TABLE 231

REPORTED INCOMPLETE INCIDENCES BY COUNTRIES OF HUMAN PSITTACOSES FROM 1931–1963 CONTACT WITH FOWL, BIRDS, AND BIRDS

Country	Pigeons C*	Pigeons D†	Ducks C*	Ducks D†	Turkeys C*	Turkeys D†	Chickens C*	Chickens D†	"Poultry" C*	"Poultry" D†	Sea-shore birds C*	Sea-shore birds D†	Total C**	Total D†	Source of Information
Argentina	?			5			10						19	6	Rugiero et al., 1950
Austria			6										6		Furst et al., 1957
Canada	1					27	1						29		Bowmer, 1958
Czechoslovakia	5		986	13 (13 per cent)		72	200		525 (geese & poultry)	1			1788	13	Strauss, 1957; Krivutka, 1959; Sery et al., 1961, and Sery, personal communication, 1962
Denmark	5 per cent to 10 per cent of atypical pneumonia														Mathleisen, 1956
Egypt	serum reactors														Zaghloul and Savel, personal communication, 1962
Faeroe Islands											186	38	186	38	Rasmussen, 1938
Finland	5.6 per cent atypical pneumonia														Jansson, 1960
France	6 per cent bronchitis														Besson, 1958
Germany (West)	30 / 51	?	2 (30)	1			15				47 (30) / 51		47 (30) / 51	1	Munnte, 1955; Fritsche et al., 1956; Kittel, 1955; Haehn (according to Moir, 1954)
Germany (East)	1								1363		1365		1365		Ortel, 1964
Great Britain	15		2								17		17		Westwood, 1953; Murray, 1960; Dow et al., 1960; Semple, 1956
Greece	1				1						2		2		Michael, 1957
Hungary	1		211 (1960–1963)				+				211		211		Dömök, 1963; Rudnai et al., 1964

[680]

plying bedsonia in the cytoplasm of a mammalian host cell.

These microscopic studies were made possible through the early discovery by Krumwiede that the agent can be transmitted and readily perpetuated in white mice.

Working with a strain of psittacosis agent well adapted to the mouse, killing them regularly in 48 hours, they studied the parasite in the spleen. About 10 hours after infection there were only large forms; at the time of the animal's death the forms were all coccal elementary bodies. There was a definite sequence of changes. When the infection had proceeded half way to death of the mouse, the infected cells showed clusters of particles of all sizes—large, intermediate, and small (elementary bodies). Some of the large forms appeared to be dividing. These observations convinced Bedson and Bland (1934) and Bland and Canti (1935) that the psittacosis agent passes through certain developmental forms and multiplies by binary fission. Burnet and Rountree (1935) and Lazarus and Meyer (1939) studying the cycle in the cells of developing chicken eggs and Yanamura and Meyer (1941) using chicken embryo cells in tissue cultures as host cells observed similar forms and sequences.

The discovery that the mouse is susceptible experimentally to the filterable infective agent of lymphogranuloma venereum (Hellerström and Wassen, 1930) led Miyagawa and his colleagues (Miyagawa et al, 1935) to demonstrate that the agent could be stained and that in size and staining reactions it closely resembles the psittacosis agent. The full extent of the resemblance was proven by Findlay and his colleagues (1938) and Rake and Jones (1942) when they showed that the lymphogranuloma venereum agent goes through a growth cycle similar in all respects to that described by Bedson and Bland (1932). Later other related agents were studied—ornithosis (Meyer and Eddie, 1942), mouse pneumonitis (Karr, 1943), feline pneumonitis (Hamre et al., 1947), and enzootic abortion of ewes (Stamp, 1951), and their cycles were similar. These studies established the common character of what became known as the psittacosis-ornithosis-lymphogranuloma, or psittacosis-ornithosis-mammalian pneumonitis (P.O.M.P.) agents (Terzin, 1958).

Of the studies that followed this period, those of Weiss (1955) deserve mention: Bland and Canti had placed the developmental cycle intracellularly, but Weiss, like Loosli and Ritter (1948), presented evidence of extracellular growth in the alveolus of mouse lungs infected with the murine pneumonitis agent.

Various aspects of the growth cycle were the subject of study, and numerous observations suggested that the psittacosis agent behaves like a true virus. It has a latent phase in its developmental cycle and changes from an infectious to a noninfectious form (Girardi et al., 1952). A restudy of the kinetic of a psittacosis infection in mice by Bedson and Gostling (1954) confirmed the observations by Heinmets and Golub (1948): the elementary bodies rapidly attach to the host cell surface and then penetrate it. Evidently during the ensuing resting phase, analogous to the lag phase during bacterial growth, the fully infectious units acquire the energy potential to multiply rapidly. When the host cell wall is finally burst, new infective units are released into the extracellular environment.

With the advent of the electron microscope and the ultramicrotome, Swain (1955), Gaylord (1954), Officer and Brown (1960), and Tajima et al. (1957) re-examined the forms developing during the growth cycles. Finally Litwin (1959, 1962) and Litwin and his associates (1961) recognized, documented, and described a simple and coherent sequence of events in the growth cycle of this group (Fig. 23.1).

Growth and Morphology

The course of the bedsonia parasitism of cells of the chorio-allantoic ectoderm or Chang's human liver cells infected with these agents is as follows:

Infection of a susceptible cell is initiated

lished (Parnas *et al.*, 1960 and 1961). In Hungary, the course of investigations of 3 outbreaks of psittacosis in poultry processing plants disclosed the following serological attack rates: outbreak I, 96 of 446 employees (21.5 per cent); II, 79 of 453 employees (17.4 per cent) and III, 30 of 852 employees (3.6 per cent). A screening test carried out on 334 employees of one plant (I) in the fourth week of the outbreak showed that 183 or 55 per cent proved negative while 151 reacted in dilutions of 1:4 to greater than 1:128; in fact, 42 or 12 per cent had CF titers greater than 1:52 (Solt *et al.*, 1962/63). Of 260 workers of poultry breeding and processing plants near Moscow, 56 or 21 per cent reacted in the CF test while 80 or 30 per cent gave positive skin tests (Il'inskii and Dareva, 1963). Another survey on 451 workers in the Sverdlovsk region in the U.S.S.R. gives similar figures with regard to the reactors; while 76 or 17 per cent were considered reactors in the CF test, 136 or 29 per cent gave allergic skin tests (Kortev and Fedorova, 1963).

In summary, the surveys briefly considered confirm the view repeatedly advanced that the reported cases of human psittacosis represent only a small percentage of the actual number of clinically mild, even inapparent, infections seen in occupations which bring man into contact with pigeons, ducks, geese, turkeys, or poultry in general in the order mentioned. Permeation of bedsonia infections on breeding farms and in processing plants may lead to subclinical attack rates of as high as 45 per cent of the employees. These facts must be taken into consideration while accepting and using the statistics presented in Table 23.1. Last of all, it is well to remember that psittacine birds continue to be responsible for sporadic and, occasionally, occupational group and family infections.

ETIOLOGY AND PARASITOLOGY

Ornithosis and psittacosis are caused by intracellular, biologically distinct parasites. These share the following characteristics:

1. They stain readily with basophilic dyes.
2. They are antigenically related according to complement fixation, immunity, and toxin neutralization tests.
3. They grow well in the yolk sac of embryonated eggs.
4. They produce, with few exceptions, pneumonitis in the laboratory mouse when introduced by the intranasal route.
5. They are susceptible to the action of certain antimicrobial drugs, particularly tetracycline.

The original studies of Bedson and Western (1930), Levinthal (1930), Krumwiede *et al.* (1930), and Sacquépée and Ferrabouc (1930) showed that their isolates from infected parrots had the following principal characters of a virus according to the views prevailing at that time:

1. Inability to multiply on the nonliving media used for bacteria and filterability through the coarser grade of Berkefeld and Chamberland filters. Filtrates of splenic and hepatic emulsions that are sterile on media used for bacteria reproduced the disease in healthy birds (parakeets and parrots).
2. Several of the first accounts made reference to very small coccal bodies resembling microorganisms which when stained with Giemsa could be seen in smears of virulent material obtained from naturally occurring human or avian cases or from experimentally infected animals by means of the ordinary microscope using white light. These bodies resembled rickettsia in their microscopic appearance. The virulence of the test material was correlated with their presence, and they were held back only by the less coarse bacterial filters. Early in psittacosis research these bodies were referred to as Levinthal-Coles-Lillie (L.C.L.) bodies.

It is to the lasting credit of Bedson (1932), Bedson and Bland (1932), and Bland and Canti (1935) that they demonstrated, through painstaking microscopic studies, that the L.C.L. bodies were pleomorphic, a constant feature of the *multi-*

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passing from one cell to another. The fluid or semifluid content of the vesicle (originally called matrix by Bland and Canti, 1935) is probably formed by enzymatic digestion of the surrounding cytoplasm by the multiplying psittacosis agent; it is rich in RNA (Pollard et al., 1960). In the trachoma and lymphogranuloma growth cycle the matrixes are unique in their content of a glycogenlike, iodophilic polysaccharide (Rice, 1936; Orfila, 1962).

The appearance of bedsonia in smears prepared from infected tissues or cultures is important. When stained by the Macchiavello method (basic fuchsin decolorized with citric acid followed by methylene blue) the dense particles and the highly infective elementary bodies show affinity for basic dyes—basic fuchsin, and stain red; the coarse reticulated forms are blue. These differences are not striking when the Castaneda procedure or the Giemsa stain is used. Neither the coarse nor the dense particles retain gentian violet, after decoloration with 95 per cent ethanol, as well as the rickettsia do. In an electron microscopic study of the structure of 15 isolates, the diameter of the individual organisms ranged from 280 to 980 mμ; the elementary bodies derived from parakeets were spherical while those of turkey, egret, pigeon, and mammalian origin were angular, suggesting a polygonal architecture (Page et al., 1961).

Biochemistry of Growth

A variety of experimental findings, reviewed by Moulder (1954), Weiss (1955), Wenner (1958), and again more recently in an admirable manner by Moulder (1962b) point to the conclusion that many members of this group are obligate intracellular parasites rich in both DNA and RNA have enzyme systems that can synthesize micromolecules of protein and nucleic acid specific for each agent. Likewise they produce metabolites of low molecular weight such as folic acid, lysine, and muranic acid, that are not synthesized by the host cells. Purified suspensions of bedsonia particles prepared from infected chick-embryo yolk

sac or allantoic fluid digested with pancreatin or cobra venom (Allen and Bovarnick, 1962) (their freedom from host cell contamination determined by serological tests and electron micrographs) have served for determination of the chemical composition. There is evidence that the two morphologic types of particles have different compositions and correspondingly different activities; the reported results are not always comparable. The psittacosis agents are chemically complex organisms. Of all the common amino acids, only arginine and histidine are missing from the parasite protein estimated at 35 per cent of the dry weight for the meningopneumonitis agent. Lysine is present, probably in the cell walls, in appreciable amounts (Jenkin, 1960).

Many members of the group contain a lipid; 6BC psittacosis strain yields lecithin (Gogolak and Ross, 1955).

About 3.5 per cent of the dry weight of the meningopneumonitis particle is DNA; the RNA varies from 2 to 7 per cent (Gogolak and Ross, 1955). The dry weight of the same organism has about 2 per cent carbohydrate (½ is hexosamine) (Jenkin, 1960).

Significant are the findings that the meningopneumonitis and murine pneumonitis particles contain the amino sugar—muranic acid—found only in bacteria, rickettsiae, and the blue-green algae (Allison and Perkins, 1960).

Nutritional requirements of the bedsoniae have been greatly clarified by intriguing studies (Morgan and Bader, 1956 and 1957) using a sensitive tissue culture system consisting of the L strain of mouse fibroblasts. Amino acid or B vitamin not required for the growth of L cells was essential for psittacosis multiplication. Four amino acids (arginine, glutamine, histidine, lysine) and two vitamins (folic acid and riboflavin) necessary for the multiplication of L cells were not required by the 6BC psittacosis agent. The first two amino acids are not found in the psittacosis agents (Jenkin, 1960). Lysine, a component of the cell wall, is formed by the meningopneumonitis agent by decar-
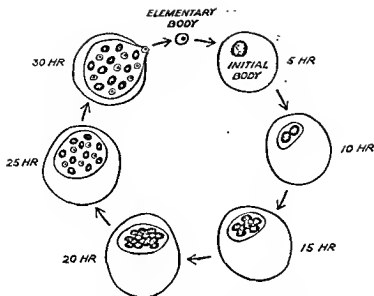
FIG. 23.1 — Growth cycle of bedsonia agents.

by an elementary body, a particle about 0.2 to 0.3μ in diameter and consisting of a limiting wall or membrane containing an electron-dense central body surrounded by less dense peripheral material. In susceptible cell cultures the elementary body becomes adsorbed to a host cell within 2 hours (Weiss, 1955; Officer and Brown, 1960). The invading body or particle undergoes an internal reorganization as it adjusts to the intracellular environment. The particle then appears as a highly granular coarse meshed reticular form surrounded by a vesicle. These larger particles (0.7 to 1.0μ in diameter) can be seen with the light microscope and have been described as initial bodies. This description is not appropriate because such units persist throughout the growth cycle, not merely at the beginning.

Between the tenth and twentieth hours after infection intense metabolic activity, but little division, is observed; the infectivity of the culture at this stage remains low and fairly constant.

After 20 hours the particles begin to increase in number, according to most evidence, as the result of binary fission of the large forms. The agent leaves the lag phase

and enters the logarithmic phase of multiplication; this is accompanied by an increase in infectivity of the culture. At a time characteristic of each host-agent system, some large forms differentiate into small dense-centered highly infective particles, and these small particles do not divide. Others of the larger reticulated particles continue to divide.

Between 25 and 30 hours division of the large forms also ceases, but differentiation of large into small forms continues for a short period. The terminal population in the host cell consists of a mixture of large and small particles. The critical analysis by Bedson (1959) and in particular the studies of Litwin and his associates (1961) on eight host-agent systems lend no support to the view that the elementary bodies develop from a viral matrix or that multiple endosporulation is an important phase of multiplication of the bedsoniae. The nature of the dense-centered bodies is unknown; they consist in large part of material other than DNA or RNA (Jenkin, 1960) and may be an aggregation or condensation of the protoplasma of the elementary body that cannot divide but can resist extracellular stresses encountered in

for 23 days. Chemical substances such as lysol, chloramine, sodium hydroxide, hydrochloric acid, and others at temperatures of 18 to 20° C. inactivated the agent within 30 minutes. Short-wave ultraviolet light inactivated the bedsonia on thin cotton material within 30 minutes from a distance of 1 m. from the source of radiation; long-wave radiation did not inactivate the agent even after an exposure of 2 hours (Bolotovskii, 1959).

Effect of Antimicrobial Drugs

Multiplication of most representative members of the group is inhibited by drugs effective against bacteria: the sulfonamides, penicillin, the tetracyclines, erythromycin, and chloramphenicol (Eaton, 1950; Hurst et al., 1953; Gale, 1959).

Bedsonia and penicillin interact in the same way as bacteria and penicillin. All members of the group are susceptible, the mammalian agents more so than the avian ones. Large, irregularly shaped and vacuolated bodies are observed when feline pneumonitis or lymphogranuloma agents are grown in the yolk sacs of chick embryos treated with penicillin (Weiss, 1955; Hurst et al., 1953; Tajima et al., 1957; Walz, 1963). These bodies, analogous to bacterial spheroblasts, remain viable and resume multiplication at a normal rate as soon as penicillin is removed by injection of penicillinase into the embryo (Moulder et al., 1956). In a manner similar to bacteria, bedsoniae become resistant to penicillin (Moulder et al., 1955; Gordon et al., 1957). The resistance develops stepwise and leads to the appearance of penicillin- and cross-neutralization-resistant mutants of the feline pneumonitis agent (Moulder et al., 1958). The peculiar properties of these mutants suggest a nondissociable bond and fundamental changes between the cell wall of the bedsonia and the drug. This led to the observation that the cell wall of the meningopneumonitis or murine pneumonitis bedsoniae is like that of bacteria and that the bedsoniae contain muramic acid (Jenkin, 1960; Allison and Perkins, 1960). This acid is synthesized by enzymes in the bedsonia and incorporated into the cell wall. Penicillin probably inhibits this incorporation and thus prevents multiplication.

Tetracyclines are currently the drugs of choice in the treatment of psittacosis (Katz, 1956; Wenner, 1958). Aureomycin did not affect the extracellular meningopneumonitis agent when propagated in the chick embryo (Allen et al., 1953); the action is directed against the intracellular growth cycle by interfering with some unknown metabolic process essential for reproduction (Moulder, 1954). Achromycin was superior to aureomycin and terramycin in delaying death of chick embryos infected with feline pneumonitis (Katz, 1956). The effectiveness was not directly proportional to the amount given but was independent of the concentration of the psittacosis agent. Achromycin administered within 48 hours after infection completely protected the embryos. After 72 hours the survival rates fell with increasing time between infection and administration of the drug. The remarkable growth inhibition by aureomycin compared with that of penicillin is clearly shown in a chart in a paper by Gogolak and Weiss (1950). Aureomycin produces smaller plaques or aggregations of particles than penicillin. Because chlortetracycline resistance developed in egg passage and in mice, Gordon and his colleagues (1957) warned that drug resistance might be produced in programs designed to eradicate psittacosis from poultry or parakeet stocks. To date drug-resistant isolates have not been obtained from avian species inadequately treated with tetracyclines for 15 to 30 days.

PATHOGENESIS

The psittacosis bedsoniae exhibit no special affinity for cells of the chicken embryo and multiply in the most readily available cells. Infected allantoic fluid, yolk sac, or mouse lung is useful for separating the elementary bodies from tissue components by procedures that include the use of proteolytic enzymes, surface active agents, absorption of cell components to

boxylation of diaminopimelic acid—a reaction not occurring in mammalian tissue (Moulder, 1962a and 1964). The lack of glutamine requirements is not understood. Folic acid is synthesized by the psittacosis group (Colón, 1960 and 1962). Cytochrome C reductase as an enzyme takes the place of nitroflavin in the meningopneumonitis agent (Allen et al., 1960). Infection causes no changes in the energy metabolism of the host cell, but alters the rate of nucleic acid synthesis. Several studies by independent workers have shown that diverse members of the group synthesize their own nucleic acid from precursors of unknown complexity within the host cells. Psittacosis agents, like bacteria and other cells, contain more than one kind of RNA, strongly indicating that the bedsoniae have synthetic systems. Quite recently careful studies by Ormsbee and Weiss (1963) with the trachoma agent have shown that purified suspensions of bedsoniae possess an independent carbohydrate metabolism in an extracellular environment; it appears that oxidation was not affected by molecular oxygen. The available evidence suggests that the psittacosis particles are active metabolic units, but there is a striking metabolic deficiency in the apparent lack of any sort of energy metabolism. Moulder (1962b) offers the explanation that the psittacosis group depends on the host cell to supply high-energy metabolites such as adenosine triphosphate and other nucleotide triphosphates that most likely penetrate the agents. Probably this deficiency explains the obligate intracellular metabolism: an adequate concentration and variety of high-energy compounds is not found outside living, actively metabolizing cells.

Inactivation

The members of this group are among the less stable infective agents under ordinary laboratory conditions. Older claims that complete destruction requires heating at 70° C. for 10 minutes must be re-examined. One of the turkey ornithosis isolates in 20 per cent mammalian tissue sus-

pension has been destroyed in less than 5 minutes at 56° C. and in less than 48 hours at 37° C. Resistance to lower temperatures is well known. When a portion of the same suspension was stored at −20° C. or at dry ice chest temperature for 400 days, the viability was gradually lost, until 99.95 per cent had been destroyed (Page, 1959b). Diseased turkeys frozen in processing plants retained the ornithosis agent in a viable state after a little over a year of storage at −20° C. or below.

A solution of 7.5 per cent dextrose in skimmed milk preserves the infectivity to a high degree whether stored after freeze drying or in a frozen state at −15° C.; lyophilized tissue suspensions yield higher infectivity than frozen suspensions (Schmittdiel, 1961). Either skim milk or glutamate medium at −70° C. preserved the meningopneumonitis agent (Allen et al., 1952).

The growth cycle is destroyed by freezing. This explains the drop of infectivity so frequently encountered when suspensions are not titrated in the fresh state (Litwin, 1959; Officer and Brown, 1960).

When preserved in 50 per cent glycerol in buffered saline (pH 7.6) and held at 0° ± 4° C., heavy suspensions of infective tissue may retain their activity for 10 to 20 days. The psittacosis agents in sputum, human lung tissue, and bird specimens rapidly lose potency in glycerol; this preservative should not be used.

Formalin (0.1 per cent) or phenol (0.5 per cent) inactivates even suspensions free from clumps in 24 to 36 hours; ether or ethanol at room temperature is destructive within 30 minutes. The pH range of stability is narrow (7 to 8), and the rate of inactivation of the feline pneumonitis bedsonia by ultraviolet irradiation is comparable to that of Escherichia coli (Moulder and Weiss, 1951).

Suspensions of ornithosis and psittacosis isolates grown in mouse brain and embryos preserved their activity in tap or well water for 17 days, in melted snow for 15 days, in natural light for 18 days, in snow in darkness for 29 days, and in pasteurized milk

Multiplication of the bedsonia is not essential for evocation of the lethal effect; it is sedimented with the infective particles when spun at 18,000 rpm for an hour, or when put through coarse Seitz filters. The lethal properties are unstable: the potency declines at 22° to 37° C. within a few hours and may be quickly lost at 44° C.; it is destroyed by 0.1 per cent formalin although the antigenicity is retained. If homologous antiserum-homogenate mixtures are administered together, the lethality is neutralized to the extent that from 80 to 90 per cent of the mice survive at least 48 hours. In the following days a variable percentage succumb to infection. Not all isolates (e.g., from severe human pneumonitis, from turkeys and egrets) may be fatal to all mice within 24 hours. The immunologic specificity of the lethality-neutralization reaction is identical or very similar to that of the infectivity-neutralization reaction (Manire and Meyer, 1950c). Recent studies have demonstrated that the toxic antigen is located in the cell wall, as is the neutralizing antigen (Ross and Jenkin, 1962). Incomplete physiologic and histopathologic studies on mice subjected to the lethal effect of bedsonia revealed profound changes in the blood elements—severe leukopenia, toxic granulation and shift to the left, thrombocytopenia and irregular marked anemia accompanied by delay in blood clotting, hemoconcentration and disturbance in the glucose metabolism. Microscopic examination of the venae cavae of mice infected by the intravenous route revealed damage to the vascular epithelia (Schoenholz, 1962). These observations suggest that the toxin of the bedsonia in some manner reflects the damage done to the host during the act of invasion of the particles. Toxin neutralization and infectivity neutralization titrate the same antigen or polymolecular substances on cell wall that is intimately involved in invasion of host cells. On the other hand, it is known that several antigens of unknown toxicity are produced during multiplication of the bedsonia (Hilleman et al., 1951; Gogolak and Ross, 1955;

Benedict and O'Brien, 1958), and that apparently the synthesis or release of a lethal toxin of a penicillin-resistant mutant of feline pneumonitis is inhibited by penicillin. Although the neutralization technique of the toxin or factors in distinguishing avian from mammalian isolates is of value, it does not seem likely that the lethal effect that follows the intravenous inoculation of mice with homogenates consisting of very large numbers of highly infective bedsonia particles explains the pathogenesis of natural infections of man and animals by members of the psittacosis-lymphogranuloma group.

Antigenic Structure

It was shown early that the sera from persons convalescent from psittacosis (Bedson, 1935) and that from diseased or recovered psittacine birds (Meyer and Eddie, 1939a) fixed complement in the presence of the psittacosis agent in the splenic tissues of mice or tissue culture. Another finding was significant: the psittacosis antigen contained two antigens, one that was heat-labile was destroyed by temperatures above 60° C.; the other withstood boiling or even autoclaving at 135° C. Immune serum contained antibodies to both antigens, as could be demonstrated by complement fixation tests with the unabsorbed serum and with those that had been absorbed separately with the whole agent or with its heat-stable component. As other psittacosis and the lymphogranuloma agents were discovered, it was found that they all have, in the complement fixation test, the same heat-stable antigen now designated the group antigen. By treating human serum from lymphogranuloma venereum by absorption with heated homologous or heterologous (psittacosis) antigen, it was found that the lymphogranuloma venereum agent contains a heat-labile fraction that is specific for this bedsonia (Bedson et al., 1949; Barwell, 1952). Antiserum in rabbits, guinea pigs, and pigeons with the enzootic abortion of ewes agent (Monsur and Barwell, 1951) and similar studies with the psittacosis and feline pneumonitis agents (Ross and Gogo-

specific antiserum or to celite and cycles of high and low speed centrifugation (Zahler and Moulder, 1953), or simply by differential centrifugation (Gogolak and Ross, 1955). Purified preparations are also obtained by dialysis against distilled water (Crocker, 1954). By use of an ingenious counting method it has been learned how elementary particles infect chicken embryos by the yolk sac route.

Counting the number of particles in suspensions of infective agents with the light microscope (Lazarus and Meyer, 1939; Gogolak, 1953; Manire and Smith, 1959; Smith and Manire, 1959) or the electron microscope (Crocker, 1954; Litwin, 1959, 1962; Jenkin, 1960; Litwin et al., 1961) and inoculating the counted suspension into the yolk sac of embryonated eggs, their invasiveness and their LD_{50} have been determined. Thus it was demonstrated that the 6BC parakeet and Borg strains of psittacosis infected in the total particle/LD_{50} ratio of almost 1; the ratio for the trachoma was close to 1×10^6 (Litwin et al., 1961; Litwin, 1962). Agents with a ratio near unity can be considered highly infectious for a variety of hosts; they invade and grow in many different kinds of cells. Bedsonia isolates or members of the group with high ratios have restricted host range and multiply in few tissues or cells. It is guessed that the structures or enzymes involved in the invasion of the host cells by the dense particles may be located in the cell wall (Jenkin et al., 1961; Ross and Jenkin, 1962).

Latency

One characteristic of natural infections with members of this group is latency without obvious harm to the host. This was first demonstrated in parakeets and mice (Meyer and Eddie, 1933; Bedson, 1938; Early and Morgan, 1946a), then in chick embryos (Davis, 1949; Greenland, 1961) and in man (Meyer and Eddie, 1951b). Latency may result from nutritional deficiencies in host cells and recrudescences of frank disease may follow when these deficiencies are corrected (Morgan and Bader,

1957; Bader and Morgan, 1958, 1961). At first glance it might seem that the cells of living intact animals are unlikely to reach a state of starvation. But the absolute and relative concentrations of metabolites within cells are subject to a variety of complex regulatory mechanisms. Even in adequately nourished mammalian or avian hosts the conditions in certain cells or groups of cells essential for bedsonia growth may fall below the minimum; the lag phase in the growth cycle normally taking about 10 hours might be prolonged indefinitely. This still has to be proved. Another method of producing experimental latency was observed by Litwin (1959) when he altered the normal growth cycle by transferring the feline pneumonitis bedsonia at the early growth stages onto chorioallantoic membranes. The cycle was greatly delayed and fully infectious particles never appeared. In this manner the agent could be transformed indefinitely in the latent phase because the rapid transfer indefinitely prolonged the early stages of normal growth. A third factor may condition latency. In Benedict's study (1958b) the psittacosis bedsonia growth cycle was greatly suppressed in monocytes derived from immune guinea pigs; they grew well in normal cells. This observation suggests that immune reactions within the mammalian or avian cells may be partly responsible for latency.

Lethal Properties

Analogous to the observation on the rickettsial toxin studies, Rake and Jones (1944), Manire and Meyer (1950 a, b & c), and Meyer and Eddie (1953) observed that the psittacosis-lymphogranuloma agents form a toxin closely associated with the infectious particles that is rapidly lethal for mice on intravenous injection. Homogenates of yolk sacs infected with a highly virulent turkey ornithosis isolate in dilution of 1:1,000 killed 50 per cent of the mice within the first 16 hours after intravenous injection. The LD_{50} of the lethal factor was more than 1×10^6 times the infection LD_{50} (Manire and Smith, 1959).

sensitized with a soluble group antigen sedimented at 100,000 G for 80 minutes from meningopneumonitis-infected allantoic fluid was agglutinated by 70 per cent of serum from patients who had had psittacosis 1 to 5 years before the test. This sensitizing antigen, probably a protein, is apparently not associated with either complement fixation activity or with the murine hemagglutinin (Benedict and O'Brien, 1958).

HOST RANGE
Natural Infections

In 1930 when the psittacosis agent was isolated and first studied, it was thought that its natural hosts were to be found only in birds of the parrot family. Of course it was known then that other species of birds were susceptible and could even be the source of human infection. Since then naturally occurring infections have been found in steadily increasing numbers of extrapsittacine birds, as the tabulations in previous editions of this book fully indicate. By the end of 1963, members of at least 127 species belonging to 10 orders had been found infected. The species extensively studied, aside from the parrots, for example the pigeons, ducks, geese, chickens, and turkeys, all showed chronic latent infections and complement fixing antibodies in the peripheral blood serum. The host range of ornithosis in wild birds is much wider than had been suspected. In the course of investigations in the Caucasus and Transcaucasia, searching for viral infections, the sera of 263 birds were studied in the direct and indirect complement fixation test. At the same time the viscera of 3,712 birds pooled in 862 batches were examined for viral agents by intracerebral and intraperitoneal inoculation of mice and repeatedly passaged agents belonging to the *Bedsoniae* were never isolated. However 28 of 196 sera reacted in the complement fixation tests in dilutions from 1:4 to 1:64. The serum of 1 of 6 bustards (*Otis tetrax* Linn. 1758) was positive in a dilution of 1:61; 2 of 24 common kestrels (*Falco timunculus*) reacted 1:32 and 1:64;

7 of 133 nestling and adult rooks (*Corvus frigelaus*) reacted in 1:16, 1:32, and 1:64 (Basova *et al.*, 1960). Other Russian investigators found the sera of 5 species of water fowl collected at the Caspian Sea (Terskikh *et al.*, 1961, 1962) and 1 of 12 cormorants (*Phalacrocorax carbosinensis*) to give positive reactions in the complement fixation test (Boldyrev, 1961).

Sparrows (*Passer domesticus*) caught on the college farm near Adelaide, Australia, yielded bedsoniae from the spleens; others trapped in the city proved uninfected (Dane and Beech, 1955). These observations suggesting that sparrows acquire ornithosis from a contaminated environment are supported by findings of organism pools of nestling birds roosting in the vicinity of diseased duck farms to be infected (Illner, 1961).

While discoveries were extending the host range of the avian agents, it became evident that mammals other than man are hosts of bedsonia species (Wenner, 1958). The knowledge by comparison with that of the avian species is limited, but the host range seems much more restricted, and what is more important, the infection more commonly results in frank, often destructive, disease involving the respiratory tract and the central nervous system and causing abortion (Meyer, 1959b).

The human host specific lymphogranuloma venereum and trachoma are members of the psittacosis group, as originally proved by Findlay and his associates (1938). The trachoma and inclusion conjunctivitis agents undergo a growth cycle as do the psittacosis bedsoniae when they multiply in the embryonated egg (Gordon, 1963; Jawetz and Thygeson, 1964).

The suggestion has been put forward that certain bedsoniae with high person-to-person communicability without apparent connection with birds have become adapted to man and are becoming so adapted that they no longer acquire an avian host. Most observers of human psittacosis had been familiar with man-to-man spread from patients in hospitals to nurses, but this has rarely gone beyond one pas-

lak, 1957a) provided evidence that the heat-labile antigen is the species-specific antigen that is always serologically weaker than the group antigen. Considerable progress has been made in identifying these antigens with chemical fractions of the bedsoniae. Treatment of the agents with phenol, dilute hydrochloric acid, or papain had the same effect as boiling; it made them group specific. Prolonged extraction with ether placed the group antigen in solution in which it could be destroyed with potassium periodate in very low concentration or by lecithinase (Barwell, 1952). In subsequent careful investigations, Ross and Gogolak (1957a) grew the psittacosis and feline pneumonitis agents in the allantoic cavity of 8-day eggs, purified them by fractional centrifugation, disrupted the particles by sonic vibration and lyophilized the particulate material. Two complement fixing antigens were extracted from these preparations, one ether-soluble, the other alkali-soluble. The latter had both the specific and the group antigen. When treated with potassium periodate or lecithinase, the specific antigen that could be destroyed with papain (but not trypsin) was obtainable. It is believed that the specific serologic activity of the bedsoniae resides in a lecithin-nucleoprotein complex. The alkali-soluble antigen fraction yields lysolecithin when treated with lecithinase and choline is demonstrable in the phospholipid fraction. Solubility of the lecithin in the alkali-soluble, ether-insoluble extract suggests that it is firmly bound to a nucleoprotein identified as such by chromatographic and spectrophotometric methods. A water-soluble group complement fixation antigen has been extracted with sodium lauryl sulfate from purified bedsoniae (Benedict and O'Brien, 1956).

A serologic procedure to detect the specific antigen in psittacosis or ornithosis isolates would be invaluable to identify its host origin. Present methods have not informed whether the extrapsittacine isolates differ sufficiently from psittacine isolates to constitute a separate species. Such a method would be used to answer urgent epidemiologic questions, for example whether ornithosis in turkeys or ducks is introduced into breeding and fattening flocks by water fowl such as sea gulls. There remains also the question concerning the variable organotropism of bovine bedsoniae—for example, "Is the bovine abortion agent merely an adaptation of the bovine enteritis agent?" This cannot be answered without an analysis of the specific antigens in the different isolates so far reported. The precise method of Ross and Gogolak is too arduous for routine use. Promising results have been reported by Jenkin (1960) and Ross and Jenkin (1962) who used cell wall preparations as antigens, treating purified bedsoniae with deoxycholate and with trypsin. Specific antigens in the cell wall were demonstrated in both direct and indirect complement fixation tests.

Slide agglutination tests with purified bedsoniae are quite useful (Lazarus and Meyer, 1939; Labzoffsky, 1947; Mason, 1959; Chang et al., 1962). Treatment of infected yolk sac suspensions with fluorocarbon and concentration by centrifugation has proved highly useful in agglutination tests in darkfield preparations (Bernkopf et al., 1960), particularly when used in fluorescent antibody techniques (Nichols and McComb, 1962).

Allantoic fluid from duck embryos infected with psittacosis, murine or feline pneumonitis, or meningopneumonitis bedsoniae agglutinates mouse erythrocytes. The serologic reaction with this so-called murine hemagglutinin is group, rather than strain, specific. The hemagglutinin consists of two chemical fractions, a phospholipid and a nucleoprotein. The former contains lecithin, which is not serologically specific but can agglutinate mouse erythrocytes. Specific hemagglutination inhibition takes place in the serum of roosters immunized with purified elementary bodies, indicating that hemagglutinins are associated with the bedsonia particle (Hilleman et al., 1951; Gogolak, 1954; Gogolak and Ross, 1955; Inaba et al., 1957).

Tannic-acid-treated sheep erythrocytes

sage. In the San Francisco and Louisiana outbreaks of severe pneumonitis the sequence of case to case transmission was considered new and striking, notwithstanding that similar transmission chains had been described in 1930 (Hamel, 1932). The course of transmission was contrary to the concept of adaptation: these strains were transmitted only from fatal cases and only late in the disease. This suggested that a strain of unusual invasiveness had met a new host for the first time. The questions of which bedsonia has adapted itself to parasitism in the respiratory tract of the human host and whether such adaptation is possible remain open. One must continue to look to birds as the source of human psittacosis with interhuman transmission (Hansen and Sørensen, 1955).

Experimental or Indicator Hosts

Table 23.2 shows the pathogenicity of bedsoniae for experimental hosts.

At least 12 years before it became known that the white laboratory mouse is a natural host of mouse pneumonitis, this rodent was found to be invaluable as an indicator host for isolation of bedsoniae. The white mouse, readily available and relatively safe with respect to infection of man, has displaced birds for experimental work, except in rare instances when Java ricebirds offer advantages. Mice highly susceptible to bacterial but not necessarily to neurotropic virus infections are preferred. Intranasal administration of an avian psittacosis agent has disadvantages in that it may become contaminated with pleuropneumonia organisms and that the mice may be latently infected with murine pneumonitis (Gönnert, 1942; Nigg and Eaton, 1944; de Burgh et al., 1945). The duration of the illness and the rapidity with which death follows inoculation depend on the amount, virulence, and toxin-producing ability of the isolate. Intracranial injection is usually fatal within 4 to 6 days when 0.03 ml. of 50 to 1,000 LD$_{50}$ is injected. Titrations of isolates from birds, given intravenously or intraperitoneally to

mice, have yielded useful information about their virulence.

When highly virulent isolates from turkeys are injected, death ensues in 2 to 6 days, with the less virulent in 8 to 15 days; some mice recover and most become carriers. In animals infected orally or subcutaneously the course is always protracted. They may excrete it in feces and urine for up to 10 days after injection; however, mice infected with a pigeon isolate did not transmit it to uninfected cagemates (Weyer and Lippelt, 1956). Latent infection has persisted for 10 to 12 months. A psittacosis agent was isolated from mice inadequately treated with antimicrobial drugs as long as 9 months after inoculation (Hurst et al., 1953).

Ornithosis, psittacosis, human pneumonitis, meningopneumonitis, opossum B, and hamster pneumonitis agents and tissue suspensions from naturally infected hosts, administered intranasally to mice, produce widespread consolidation of the lungs (Hornus, 1940; Gogolak, 1953; Kovac, 1961). The psittacosis infection of the murine lungs begins as an unspecific irritation of the alveolar epithelium during multiplication of the agent. Then there follows sudden onset of specific proliferation within the alveolar wall, exudation into the alveolar cavity, and unsuccessful repair leading to fibrosis of the walls. Intranasal infection is invariably accompanied by perivascular infiltrations, granuloma formation, and necrosis in brain, heart, liver, spleen, kidneys, and suprarenals as a sequel to bedsonemia, infecting tissue descendants of ecto-, ento-, and mesoderm germ layers (Kovac, 1961). Discrete foci of pneumonia are manifested as limiting infective dilutions of the agents are approached; the areas are gray, almost translucent, and are 1 to 3 mm. in diameter (Loosli et al., 1948; Marinescu et al., 1960).

After they have been adapted to survival in the mouse, other bedsoniae induce fatal pneumonitis. Intracranial injection causes irritability, ataxia, convulsions, and death

TABLE 22.2

Schematic Outline of Pathogenicity of Bedsoniae for Experimental Hosts

STRAIN OF VIRUS	ORIGIN	MOUSE	GUINEA PIG	HAMSTER	COTTON RAT	CAT	RABBIT	MONKEY	PARA-KEET	RICE BIRD	PIGEON	EGG YS	TOXIN
MAMMALS													
LGV	MAN											+	‡
SF PNEUMONITIS	MAN											+	‡
LOUISANA PNEUMONITIS	MAN											+	‡
ILLINOIS PNEUMONITIS	MAN											+	‡
MENINGOPNEUMONITIS	?											+	‡
MURINE PNEUMONITIS	MICE											+	‡
FELINE PNEUMONITIS	CATS											+	‡
SHEEP PNEUMONITIS	SHEEP											+	‡
ENZOOTIC ABORTION EWES	SHEEP											+	‡
OPOSSUM A ENCEPHALITIS	OPOSSUM											+	‡
OPOSSUM B ENCEPHALITIS	OPOSSUM											+	‡
BOVINE ENTERIC VIRUS	CATTLE											+	‡
BOVINE ENCEPHALOMYELITIS	CATTLE											+	‡
HAMSTER	?											+	?
BIRDS													
PSITTACOSIS — PSITTACINE (PARAKEETS & PARROTS)												+	+(+)
PSITTACOSIS — PARROTS (HUMAN)												+	+(+)
ORNITHOSIS — PIGEON												+	+(+)
ORNITHOSIS — CHICKEN												+	‡
ORNITHOSIS — DUCK												+	‡
ORNITHOSIS — TURKEY												+	‡
ORNITHOSIS — EGRET												+	‡
ORNITHOSIS — FULMAR												+	?
ORNITHOSIS — SEAGULL													

IP = INTRAPERITONEAL
IC = INTRACRANIAL
IN = INTRANASAL
IV = INTRAVENOUS
SC = SUBCUTANEOUS
EB = ELEMENTARY BODIES
O = ORAL

TYPICAL LESIONS - FATAL
FATAL TISSUE AFTER ADAPTATION
INCONSISTENT ILLNESS AND DEATH
CARRIER STATE, ANTIBODIES
CARRIER STATE, RECOVERY AND IMMUNE
FEVER, ANTIBODIES
NO APPARENT EFFECT

turkey isolates by the intratracheal route; the resulting infection was fatal to 25 per cent of the birds (Pate et al., 1954). Parakeet and pigeon isolates and turkey isolates of low virulence inoculated into turkey poults by the same route produced neither death nor even illness, but specific antibodies appeared and the isolates persisted in the organs for 3 months or longer (Davis et al., 1958).

Virulence

This term is used in a relative sense to define the ability of different strains, injected with a syringe, to produce death from psittacosis or ornithosis in laboratory mice or avian species measured in their LD_{50}. The test measures the artificial virulence of a bedsonia for an animal species. It provides inadequate information about the pathogenicity for certain species since pathogenicity, aside from virulence, takes into consideration the invasiveness and communicability, those ill-defined characteristics that are involved before the agents are established in the tissues or blood stream. Differences in the observed infective capacity or virulence of individual strains or isolates of the bedsonia group on application to the tissues of certain hosts have been noted since the agents were first studied. Meager is the knowledge of pathogenicity or the disposition to virulence of the species and varieties within the bedsoniae. Isolates from human victims with severe psittacosis and from the psittacine bird that was the source of the infection have exhibited a high virulence for various indicator hosts on initial test infections on mice, ricebirds, or the chick embryo. Benign psittacosis observed here and abroad during the past 20 years among persons directly or indirectly exposed to pigeons, chickens, and ducks was, as a rule, caused by strains of low virulence for mice even after adaptation. In a study on the epizootiology of psittacosis in Australia, generally overlooked, Miles (1959b) refers to an important observation made in 1953, that a bedsonia isolated from the enlarged spleen of a sparrow (Passer domesticus) was

of such attenuated virulence for mice it could be isolated only in the yolk sac of the chick embryo. This must have been a unique observation, since Page and Bankowski (1959) in California and Illner (1961) obtained from nestling sparrows caught in East Berlin bedsoniae that infected mice with typical lesions in the second passage. Two significant observations have been made with the isolates from turkeys. Severe occupational human infections in Texas, New Jersey, and Oregon following temporary close association with grossly diseased turkeys were caused by bedsoniae of high virulence for all indicator hosts (Meyer and Eddie, 1954a). On the other hand, the processing of a flock of turkeys that had extensive gross visceral lesions caused no human infections. The bedsoniae isolated from these diseased turkeys when first isolated and even on passage remained of low virulence for mice (Meyer and Eddie, 1956b). Similar observations have since been made by others in Wisconsin and Minnesota, California and Oregon (Graber and Pomeroy, 1958; Gale, 1960; Page and Bankowski, 1959; Page, 1960; Meyer and Eddie, 1959, 4th ed., p. 526). Already in 1952 serologic surveys conclusively proved that ornithosis in turkeys though widely distributed in the United States had caused few serious explosive outbreaks of human illness. It was the epidemiologic study in Selma, California, that demonstrated that isolates of low virulence for mice are responsible for the serum reactions and the anatomical lesions on the viscera of the turkeys. Though of low artificial virulence in laboratory tests, the California and Wisconsin isolates have wide communicability, as attested by the high infection rate in the flocks. Identical conditions are fully recognized in ornithosis of pigeons. Subsequent investigations in California confirmed these facts, but brought others to light when the isolates from two flocks of diseased turkeys were studied carefully. Both isolates equally virulent for the chick embryo and mice differed greatly in their pathogenicity for pigeons and turkeys. One

within 3 to 6 days. The meninges are moist and deeply injected. Microscopically the meningo-encephalitis is characterized by an exudate of polymorphonuclear and mononuclear cells, rich in elementary bodies, extending along the blood vessels into the brain. This route is useful to increase the amount of bedsoniae in a clean, readily accessible form and to demonstrate quickly the specific particles, provided the material is free from contaminants and is relatively rich in elementary bodies. Irradiation with ultraviolet rays or cortisone alone or in combination failed to heighten susceptibility in pigeons, but whole body irradiation as well as histamine did render the birds more susceptible (Strauss and Vecerek, 1961). In fact, in a dosage of 0.05 mg. to 1.0 mg., histamine phosphate has activated some carriers to shed bedsonia (Arnstein, 1963).

Among the experimental hosts used to study the pathogenicity of a bedsonia, the guinea pig occupies a prominent position. It is used to isolate mammalian (bovine) bedsoniae, to determine the virulence of an isolate, and to provide high-titered antibodies for immunity studies.

In guinea pigs most isolates produce only a prolonged febrile illness, if that, when inoculated intraperitoneally. But the toxic Borg pneumonitis, egret, turkey, and mammalian (bovine enteritis and encephalomyelitis and pneumonitis of goats and sheep) bedsoniae are highly virulent. The animals succumb to large amounts of inoculum within 6 to 10 days after a febrile course of 3 or 4 days, with temperatures as high as 41.4° C., visible illness, weakness, and progressive emaciation. At necropsy, enlargement of the spleen is usual; a mucoid, viscous, stringy exudate covers the organs, and in a few animals pulmonary consolidation involves one or all lobes of the lung. Occasionally pigeon and duck isolates may cause fatal infection on intraperitoneal injection (Strauss, 1956).

Some isolates produce fatal meningo-encephalitis in rabbits infected by the intracerebral route; occasionally extensive pneumonic consolidation is produced by

intratracheal injection. Infection of the rabbit's eye has produced a violent pan-ophthalmitis (Evans and Moore, 1950).

Cotton rats are highly susceptible to the Borg isolate by the intraperitoneal, intra-nasal, or intracranial route. Wild and white laboratory rats and deer mice are not very susceptible. Syrian hamsters and squirrels (Citellus beecheyi) may be fatally infected with certain turkey isolates by the intranasal or intracranial route. Macacus rhesus may be infected by the intracerebral route; psittacosis and highly toxic turkey isolates produce typical pulmonary lesions (Rivers and Berry, 1931; McGavran et al., 1962). A parrot isolate inoculated intra-cerebrally led to meningo-encephalitis (Rivers et al., 1931).

The lesions produced in the rhesus monkey by small particle aerosol exposure have been discrete and never fatal (McGavran et al., 1962; Berendt et al., 1962).

Parakeets from aviaries free from psittacosis are susceptible to intramuscular, intranasal, or intracranial infection. Immature birds readily contract the infection by exposure to sick birds shedding bedsoniae in droppings. When death occurs during the acute stage, gross findings are a semipurulent coating over the air sac and the inner lining of the sternum, exudate in the pericardial sac, a large liver occasionally studded with areas of necrosis or infarction surrounded by hemorrhagic zones, a large spleen sometimes unevenly spotted and large soft kidneys; only rarely are lesions demonstrable in the lungs (Moritsch and Kovac, 1956).

Pathogenicity tests on pigeons by intracerebral inoculation may be useful. As a rule, isolates from pigeons, but not from other birds, produce meningitis and fatal paralysis (Pinkerton and Moragues, 1942; Strauss, 1956). Except for the meningo-pneumonitis and one murine pneumonitis isolate (de Burgh et al., 1945) no tested mammalian isolate has been pathogenic for ricebirds, parakeets, or pigeons by any route of inoculation, even with very large doses.

Turkey poults have been infected with

turkey isolates by the intratracheal route; the resulting infection was fatal to 25 per cent of the birds (Pate et al., 1954). Parakeet and pigeon isolates and turkey isolates of low virulence inoculated into turkey poults by the same route produced neither death nor even illness, but specific antibodies appeared and the isolates persisted in the organs for 3 months or longer (Davis et al., 1958).

Virulence

This term is used in a relative sense to define the ability of different strains, injected with a syringe, to produce death from psittacosis or ornithosis in laboratory mice or avian species measured in their LD_{50}. The test measures the artificial virulence of a bedsonia for an animal species. It provides inadequate information about the pathogenicity for certain species since pathogenicity, aside from virulence, takes into consideration the invasiveness and communicability, those ill-defined characteristics that are involved before the agents are established in the tissues or blood stream. Differences in the observed infective capacity or virulence of individual strains or isolates of the bedsonia group on application to the tissues of certain hosts have been noted since the agents were first studied. Meager is the knowledge of pathogenicity or the disposition to virulence of the species and varieties within the bedsoniae. Isolates from human victims with severe psittacosis and from the psittacine bird that was the source of the infection have exhibited a high virulence for various indicator hosts on initial test infections on mice, ricebirds, or the chick embryo. Benign psittacosis observed here and abroad during the past 20 years among persons directly or indirectly exposed to pigeons, chickens, and ducks was, as a rule, caused by strains of low virulence for mice even after adaptation. In a study on the epizootiology of psittacosis in Australia, generally overlooked, Miles (1959b) refers to an important observation made in 1953, that a bedsonia isolated from the enlarged spleen of a sparrow (Passer domesticus) was

of such attenuated virulence for mice it could be isolated only in the yolk sac of the chick embryo. This must have been a unique observation, since Page and Bankowski (1959) in California and Illner (1961) obtained from nestling sparrows caught in East Berlin bedsoniae that infected mice with typical lesions in the second passage. Two significant observations have been made with the isolates from turkeys. Severe occupational human infections in Texas, New Jersey, and Oregon following temporary close association with grossly diseased turkeys were caused by bedsoniae of high virulence for all indicator hosts (Meyer and Eddie, 1951a). On the other hand, the processing of a flock of turkeys that had extensive gross visceral lesions caused no human infections. The bedsoniae isolated from these diseased turkeys when first isolated and even on passage remained of low virulence for mice (Meyer and Eddie, 1956b). Similar observations have since been made by others in Wisconsin and Minnesota, California and Oregon (Graber and Pomeroy, 1958; Gale, 1960; Page and Bankowski, 1959; Page, 1960; Meyer and Eddie, 1959, 4th ed., p. 526). Already in 1952 serologic surveys conclusively proved that ornithosis in turkeys though widely distributed in the United States had caused few serious explosive outbreaks of human illness. It was the epidemiologic study in Selma, California, that demonstrated that isolates of low virulence for mice are responsible for the serum reactions and the anatomical lesions on the viscera of the turkeys. Though of low artificial virulence in laboratory tests, the California and Wisconsin isolates have wide communicability, as attested by the high infection rate in the flocks. Identical conditions are fully recognized in ornithosis of pigeons. Subsequent investigations in California confirmed these facts, but brought others to light when the isolates from two flocks of diseased turkeys were studied carefully. Both isolates equally virulent for the chick embryo and mice differed greatly in their pathogenicity for pigeons and turkeys. Our

(so-called C_2 Woodland) was at least 30 times more virulent for turkeys than the other isolate (C_4 Modesto); the latter isolate was 525 times more lethal for pigeons on intraperitoneal inoculation (Page, 1960). These important observations deserve confirmation before they are used to speculate on the ecology of the bedsoniae in turkey flocks. However, it is permissible to conclude that ornithosis of turkeys may be caused by bedsoniae of high or low virulence for mice. The latter variant is unquestionably responsible for the serologic reactions encountered in many flocks that show no abnormal mortality.

From experience with other infective agents it might be assumed that a bedsonia of low or attenuated virulence would survive best in its natural host. There are some indications that this assumption holds true for certain pigeon and duck isolates and to some extent of isolates from apparently healthy adult turkey carriers. Some from pigeons, even after repeated passage, retained their low virulence rarely killing more than 10 per cent of the intraperitoneally inoculated mice within 12 days after the inoculation. However, with some isolates from pigeons the mortality rate is 80 per cent in mice. Experiments using the natural host would require squabs or poults of uniform genetic background. Incidental observations indicate that in the "neutral host," the embryonated egg, some isolates are quite stable, others are labile and lose their virulence for the mammalian indicator host. The entire problem of virulence, and especially of changes in virulence under natural conditions, is of greatest importance and is basic to understanding the host-parasite relationship.

In this connection several observations on psittacosis in parrots deserve mention. During severe widespread epizootics in wild parrots of several species in Australia, the isolates by Burnet (1935) were apparently of heightened virulence only for parrots (Burnet, 1939). No unusual characteristics were discernible in their behavior after mouse inoculation and no human

cases were reported from the affected districts. There is no evidence on whether the epizootic bedsonia was a variant from the same host with enhanced virulence, invasiveness, and communicability, or whether it originated from another species of bird. During a survey of four parrot species made in South Australia (Miles, 1959b), 45 per cent of the Adelaide rosella (Platycercus elegans Adelaide) yielded a psittacosis agent from their viscera; the epizootic was not fatal and did not affect Kakatoe roseicapilla or Psephotus haematonatus commonly seen in close contact in the same tree. Nothing is known about the virulence for mice of the isolates from the rosellas, but the low communicability both for psittacies and such gregarious birds as starlings and pigeons suggests that bedsoniae of low virulence are more prevalent than those of high virulence. Already in 1934 it was pointed out that the psittacosis agent prevalent in recently captured budgerigars was of low virulence but became highly communicable and virulent when maintained in captivity under crowded conditions of aviaries.

The virulence test on the laboratory mouse has been used as a means to gauge the infectivity and pathogenicity of ornithosis strains in poultry. Titrations of isolates in the first or second passage given intraperitoneally to mice have yielded useful information. Repeated titrations reveal differences between log ID_{50} and LD_{50} and these may be used to calculate a pathogenicity index (Page, 1959c). As the lethality titer approaches equivalence with the infectivity titer the index approaches 0. A low index indicates high mouse virulence and a high index (3 to 4) indicates an infectivity 10,000 times greater than the lethality. In general isolates from human infections, parrots, many parakeets, and turkeys involved in single cases and in outbreaks have yielded a low index. Most isolates from pigeons, ducks, and a few from turkeys and parrots have yielded high indices. For example, the New Jersey turkey isolate with an index of 0.34 was responsible for 17 human infections; the

Selma isolate with an index of 1.92 caused no infections among 88 employees. Monreal (1960) modified the Page pathogenicity test and applied it to two isolates from human sputum and three isolates from spleen samplings of parakeet aviaries. He determined the log ID_{50} merely by gross and microscopic examination, not by subinoculations of the organs of the mice that were sacrificed on the 21 days after inoculation. In this respect his method differs from that of Page. It may yield slightly different indices since two of the parakeet isolates had lower pathogenicity indices than the human sputum isolates. One must agree with Monreal that his procedure does not appraise the human pathogenicity of isolates from parakeets. Numerous tests continue to show that turkey and isolates with a low index are as a rule involved in the epidemic episodes in processing plants, while with sporadic cases the index is above 2. For the sake of uniformity, dilutions of the isolate should be made with second or third lung passage material from mice infected intranasally after enrichment of the agent by intraperitoneal inoculation. The tests should not be made with isolates adapted to mice after initial yolk sac propagation.

TAXONOMIC POSITION OF THE GROUP

The taxonomy of this group has been critically reviewed by Weiss (1955). Bedson (1959), on the basis of his extensive experiences, also analyzed the evidence on which a taxonomic position could be decided. He came to the conclusion that these agents are neither large viruses nor small rickettsiae. They occupy a distinct position between the two. Incidentally, one characteristic that distinguishes the rickettsiae from the psittacosis agents is their occurrence in various arthropods under natural conditions. Experiments are now being made with feather mites, collected on chicken and turkey ranches having yielded isolates of variable virulence, to learn whether the insects are true hosts or merely passive vehicles of the agent in the environment of the poultry for many months (Eddie *et al.*, 1962). A few years ago Bedson (1959) concluded from his own studies and the observations of others that the psittacosis-lymphogranuloma venereum-trachoma group of infective agents have characters both of viruses and of rickettsiae, so providing a link between these microbial orders. Since then the superb studies of the Moulder school at Chicago and Camp Detrick clearly show that members of the group structurally, biochemically, and functionally are certainly not viruses, but bacteria adapted to intracellular parasitism. However, it must be reserved for the future to determine their true taxonomic position in the microbial orders.

ECOLOGY OF AVIAN INFECTIONS

The ecology of psittacosis in birds of show and pleasure, particularly in commercial breeding and raising establishments and in pet shops, has been elucidated, documented, and described (Meyer, 1942; Meyer and Eddie, 1958c). The infective agent is passed from parent birds to the offspring while they are closely associated; in parakeets while the young birds are still in the nest. Some become ill, a small percentage (up to 10 per cent) die, but the remainder recover slowly and in due course seem quite healthy. If stress of crowding, traveling, or unsuitable diet upsets the host-parasite balance, they may become ill with ruffled feathers and diarrhea. The bedsoniae are profusely shed in the environment and any cagemate not previously infected contracts psittacosis. Recovery is rarely complete and the chronic carrier stage in about 10 per cent maintains the infection in the breeding flock. In poorly managed aviaries, psittacosis has until recently been enzootic in the majority of domiciliated caged psittacine species. There is increasing evidence that the infection does not perpetuate itself in the wild psittacine birds through nest infection.

Modern air transportation enables the investigator to procure parrots directly from the wild. With the aid of serologic tests he can prove that many shipments

are entirely free; others may contain very few psittacosis carriers and shedders. During captivity over several months the infective agent may spread from the carrier and cause general illnesses, even loss of the entire shipment. Limited observations made on wild budgerigars (*Melopsittacus undulatus*) throughout Australia have shown that they were not infected. These findings on merely 28 birds differ from those recorded on the same species held in captivity. It raises the possibility that parakeets in the wild state are largely free from psittacosis and the agent is perpetuated through nest infection in rare instances. On the other hand there is good evidence that fatal and nonfatal epizootics of psittacosis occur in Australia (Burnet, 1939; Miles, 1959b) and South America (Parodi and Silvetti, 1916). How to explain these observations is quite difficult. Under aviary and pet shop conditions an epizootic of psittacosis affects most if not all the species, although there may be differences in susceptibility between different species. Evidence for such cross infection is not easy to obtain in the wild. Rarity or absence of infection in certain psittacine species or gregarious birds suggests that it cannot occur commonly except when a highly virulent agent spreads from rosellas to gray parrots as in 1938 to 1939.

Under natural wild conditions cross infection or transmission from one species of bird to another may be expected to take place only provided an infecting strain of very unusual virulence and transmissibility is epizootic (Miles, 1959b). Whether this interpretation is correct requires more searching and sophisticated ecologic studies than have been reported. Between 1909 and 1910, South American and Australian importations of parrots were made by sea voyages lasting for weeks, often after a prolonged holding period after they had been caught. Invariably such shipments consisted of a variable percentage (up to 55 per cent) of diseased and infected birds with ample evidence of cross infection (Meyer and Eddie, 1939b; Meyer, 1962). The epizootics were man-made and in no

way reflected the ecologic conditions as they occur in the wild.

At first it appeared that the fulmar ornithosis on the Faeroe Islands had been introduced. The human disease had not been seen on the islands before 1930, and bedsoniae could not be detected in Shetland Island fulmars from which the Faeroe colony originated (Miles and Shrivastav, 1951). The severity of disease and high mortality among the women victims suggested that a psittacine, instead of nonpsittacine, bedsonia was involved. Rasmussen (1938) suggested that infection of the Faeroe fulmars came from dead parrots thrown overboard from vessels bringing the birds to Great Britain and western Europe in 1929 to 1930. This speculation must be accepted with reservations since many water birds are naturally infected with bedsoniae of virulence comparable to that incompletely studied by Haagen and Mauer (1938); the serologic examination on the Shetland Islands was done on only 12 fulmars by the direct complement fixation test. This test may not have sufficed to detect an existing infection. The stable association of birds with bedsoniae extends over such a wide geographic area and involves so many species that it is hard to imagine that it has existed only the length of time it has been recognized. Maintenance of the bedsoniae is assured by the flocking and nesting of birds. Fulmars, petrels, mutton birds, domestic and feral pigeons congregate and nest together. They become hosts and maintain ornithosis indefinitely. Bird species of solitary habits are rarely if ever found infected with bedsoniae.

Enzootic ornithosis in pigeons has been conclusively proven by many studies in many places; it is latent and inapparent, producing no visible symptoms and no pathologic lesions beyond an enlarged spleen or pericardial lesions. There is conclusive evidence that the bedsoniae are transmitted in the nest. Young birds of high susceptibility may succumb, others recover and become immune, and some become carriers that shed the ornithosis agent

in the droppings; during raising of the young it is present in the crop content. Environmental factors may favor either host or parasite. If for some reason a high proportion of the nestlings escape infection, they may contract it later and succumb. Disturbances in the environment such as exceptional weather, low temperature, crowding in unsanitary quarters, and improper feeding induce relapses of the chronic infection. Wherever the ornithosis agent is dispersed in large quantities and the pigeons are held in crowded quarters, the spread from diseased to susceptible is rapid. The apparent stability of the relationship between the pigeon host and the bedsonia suggests that it is not of recent origin. The ornithosis agent with few exceptions is of relatively low virulence.

When ornithosis was discovered in chickens, ducks, geese, turkeys, and pheasants on game farms, the question arose as to how incubator-hatched poultry became the host to the ornithosis agents. Nest infections of the sort proved for psittacine birds and pigeons could not be proved, and egg transmission in turkeys could not be found (D. E. Davis et al., 1957a; Page and Bankowski, 1959). The ornithosis agent was transmitted through the eggs of ducks and the black-headed gull (Illner, 1962a, Lehnert, 1962). The infective agent may in some way persist on the farms, either in adult birds or in the soil or bedding of the environment, or be introduced from wild birds. Present knowledge is meager because poultry pathologists and epidemiologists have not studied the life history of large poultry flocks from incubator-hatched eggs through egg-laying maturity. As a source of the infection of poultry with the ornithosis bedsoniae, Oriel (1964) suspects a homogenous-heterogenous infection chain which he expresses as follows:

Wild bird species ← Poultry → Poultry →

(sea gulls, petrels,   (ducks, geese,
feral pigeons,   chickens,
herons, sparrows,   pigeons, and
migratory birds, etc.)   turkeys)

↓   ↓

Man   Man → (Man)
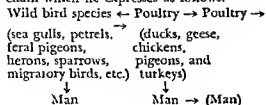
Pigeons

These fall into four groups—poultry pigeons, carrier and racing pigeons, fancy pigeons, and feral pigeons. Their wide distribution has made them readily accessible subjects for study. Pigeons, probably originally the rock dove in Europe, have been partially domesticated and carried to all parts of the world. They have adapted to living in close association with man, and they are readily bred in lofts to produce a variety of plumage or to provide squabs for the table. Their homing instincts are used for practical purposes as carrier or racing pigeons. Terhaag (1956) described how transportation of racing pigeons in baskets by train from Germany to southern France for the racing season and the sudden release of 10,000 to 20,000 birds created infective dust and spread the infection. In West Germany breeders of over 100,000 racing pigeons have created an epidemiologically important reservoir. The demand for squabs as luxurious table birds has led to high production on certain farms, sometimes housing as many as 5,000 breeding birds. The flocking and nesting of wild pigeons through all months of the year bears directly on the persistence of ornithosis (D. J. Davis, 1955; Shaughnessy, 1955; Fritzsche et al., 1956).

An excellent description of ornithosis in a self-contained pigeon flock at the Field Station of the Agricultural Research Council in Great Britain was given by Hughes in 1947.

The distribution of the infection within different age groups is significant. It mainly affects birds under 16 weeks of age. Of the 16-week-old squabs, or squeakers, as many as 20 per cent either may die or, when sacrificed, may show lesions of ornithosis. When processed for the market they may show extensive plastic exudates on the air sacs and over the heart and liver (Hughes, 1947). So-called wet squabs suffering from diarrhea may be affected with either ornithosis or salmonellosis. The incidence of these two infections was 60 and 62 per cent respectively in 552 birds

The known and reported incidence and geographic distribution among domesticated and wild pigeons, according to serologic surveys and bedsonia isolations, was first published in the 1959 edition of this book. At that time 18 countries on every continent had listed a varying percentage of reactors and isolations of the agent causing columban ornithosis. Now the number of countries where this infection has been recognized has risen to 25. Additional surveys have not been made in the United States. A summary is included in Table 23 3.

Ornithosis is a cosmopolitan, enzootic, principally inapparent infection in domesticated and feral pigeons. According to serologic surveys the proportion of reactors may range from 10 to 80 per cent, depending on the age of the birds and the complement fixation technique used. The percentages of reactors in adult birds may be higher when the indirect test is used. Isolation of bedsoniae from reactor birds has been successful in about 40 per cent of the attempts. Bedsoniae have been isolated from pigeons with no complement fixing antibodies (Meyer, 1941; Terzin et al., 1957); at the time of death, the only evidence was a slightly enlarged spleen. The endemicity among feral pigeons is roughly the same in Chicago, Baltimore, and New York as it is in Paris, Geneva, Hamburg, and Liverpool.

Certain commercial squab farms under excellent sanitary management have had much lower carrier rates and correspondingly fewer mortalities among squabs than the poorly run lofts of occasional breeders or fanciers.

In Holland, with a million people, ornithosis is widespread. In the course of examination of about 20,000 domesticated pigeons, Jansen (1955), director of the Pigeon Health Service at the Institute for Infectious Diseases at the state university at Utrecht, in 1954 found evidence of the infection in 16.4 per cent of 2,637 pigeons brought to the clinic and examined there on account of visible illness. Respiratory symptoms and conjunctivitis were pre-

eminently associated with ornithosis in racing pigeons; white fancy pigeons seem more likely to show intestinal disturbances. A sharp seasonal rise in September and October coincided with the beginning of the racing and exhibition season when large numbers of young birds came together for the first time. In isolated small flocks with a few carriers the incidence of acute disease was usually lower. A selection of 504 racing pigeons was made from 102 flocks during the winter months when they were not travelling and acute infection could be excluded. The sera of these birds were examined in the direct complement fixation test in the presence of bedsonia antigen. Accepting a titer of 1:32 and higher as indicative of infection, 77 (75 per cent) of the flocks were infected. If titers below 1:16 were considered, an additional 8 (7.8 per cent) were listed as suspicious; only 17 (16.67 per cent) of the flocks were free from ornithosis (Monreal, 1958).

In the United States infections have relapsed and become acute in pigeons fed thiamine-deficient diets (Pinkerton and Swank, 1940) or housed unhygienically (Meyer and Eddie, 1942). An outbreak of ornithosis (Smadel et al., 1943a) and an epizootic in a pigeon platoon (Smadel et al., 1945) disclosed a new virus, immunologically different from the psittacosis bedsonia, that produced intranuclear herpeslike inclusions and local necrosis of parenchymatous tissues. The malady at first affected adult birds, but later was limited to birds 1½ to 5 months old.

In other studies of lofts where pigeons have died of ornithosis, concurrent salmonellosis has been found. It is then impossible to determine which infective agent caused death.

Ducks

Ornithosis has been enzootic and for the most part clinically inapparent in ducks in the United States (Meyer and Eddie, 1953; Korns, 1955).

A bedsonia of low virulence for mice and causing a carrier stage in pigeons on intra-

TABLE 23.4
Incidence by Countries of Ornithosis in Ducks or Geese
(Published Reports 1945-1961)

Place	Reports by Authors*	Year	Serological Tests — Total ducks or geese tested	Reactors in CF and ICF tests — Tested	Pos.	Per cent	Virus Isolations — Attempted	Isolated	Per cent
UNITED STATES									
California	Meyer & Eddie (1952)	1945	1 duckling	65	32	40.2	11	1	18.0
		1946	11 ducklings	15	8	55.0	116	2	34.5
		1955 (1951)	116	39	23	59.0	97	40	31.0
Long Island, N.Y.	Korns (Meyer & Eddie)	1945 and Oct. 1951	97				112	31	34.0
Several farms in Chicago	Jacobs (1957)						1955—14	4	28.0
							1956—35	3	8.5
							7	2	
CZECHOSLOVAKIA									
General	Strauss	1956							
East Bohemia	Sery, Strauss, Fre, & Klenhauer	1957	46	(14)		(30.4)			
Bohemia	Strauss, Fre, & Sulcová	1958	151 geese	(58)		38.0			
Moravia	Strauss, Fre, & Sulcová	1958	743 at random (24 farms)	336		45.2	43	15	34.8
East Bohemia	Kominik	1960	25	8		32.0	(314)	6	
East Slovakia	Strauss & Restetter	1960	314 samples from 78 area						
East Slovakia	Polony, Koppel, & Vrriak	1960	8	3	3	37.5			
AUSTRIA	Furst, Kovac, & Morusch	1957	1852 (60 flocks)	490		25.0			
	Thamm (1964)	1962 (Aug–Sept.)	2629 (Same flocks)	233	2		71	24	33.0
GERMANY		1962 (Nov–Dec)	812 (35 farms)			28.6	Mature breeding 19	1	5.0
	Lehnert & Hille	1960					immature breeding 1–4 days old—25	2	8.0
	Iliser (1926b)	1962					5–8 wks old—27	21	77.0
POLAND	Parnas, Szmunes	1961	416						
	Parnas	1964	926	18	18	1.9	5 geese	5 geese	1
ROUMANIA	Sarateanu, Nastac, Fuhrer, Oprescu, & Hung	1960							

Parentheses ( ) — Not verified

* Year of publication indicated when different from year of observation or when a choice of publication for that year is needed to identify.

cerebral inoculation was recovered from ducks of all ages, the youngest only 4 days old. Sick or dead ducks from flocks suspected to be infected were tested and about 30 per cent yielded bedsoniae. In one flock ducklings (54 per cent) had gross anatomical lesions, and the mortality rate was higher among ducklings than adult birds. Newly hatched ducklings probably contract the infection when brought to unsanitary premises where infected adults are kept. Since only very few duck farms have been conspicuously involved with human infections, the incidence is not known. Coexistence of ornithosis with duck virus hepatitis, salmonellosis and pasteurellosis may complicate an epizootiologic picture (Meyer and Eddie, 1952; Korns, 1955).

Early serologic studies (Eddie and Francis, 1942) in Michigan were indirectly confirmed when investigators in Chicago discovered that 5- to 8-day-old ducklings received from a neighboring state were infected with a bedsonia. The ornithosis infection apparently conferred a marked resistance to a challenge dose of *Plasmodium lophurae* malaria parasites. The resistance was associated with increase of reticuloendothelial elements in the enlarged liver and spleen due to the ornithosis infection (Jacobs, 1957).

Interest in ornithosis of ducks has shifted to Europe. Since the reports are not available in English, some details are given. In 1948 Terskikh (1957b) in the U.S.S.R. studied 5 outbreaks of human psittacosis in poultry processing plants where Peking ducks that looked normal were defeathered and eviscerated.

Bedsoniae were isolated from the liver and spleen of 3 of 8 ducks from a breeding farm in Austria; the isolates on intraperitoneal inoculation of the duck tissues caused death of the mice on days 4 to 6. Microscopically the agent could not be distinguished from a parakeet bedsonia. Cleaning of the duck pens had caused clinical pneumonitis in 6 attendants serologically diagnosed as psittacosis (Fürst *et al.*, 1957).

A steadily increasing incidence of atypi-

cal pneumonia among employees of poultry farms in Czechoslovakia since 1949 led to a systematic, comprehensive, and exceedingly well-conducted investigation of ornithosis in ducks. Isolation of a bedsonia from sputum of an infected poultry feeder in 1953 (Trojan and Strauss, 1955) led to investigation of 104 cases of pneumonias solely among workers in a processing plant engaged in plucking ducks and geese. Over 80 per cent of the patients were pluckers; others fed and killed poultry. In 1955, epidemic and epizootic ornithosis broke out in Bohemia and in Moravia; in a district in central Bohemia, 600 of 2,000 ducklings died. Two bedsoniae were isolated (Strauss, 1956). Further isolations were made in connection with outbreaks among dairy and poultry farmers who came in contact with newly hatched infected ducklings. In 3 of 35 apparently healthy mature ducks, which in the summer of 1955 had shown clinical signs of acute ornithosis, the bedsonia was isolated during the summer of 1956 (Strauss, 1957).

In the course of these studies a bedsonia was isolated from black-headed gulls (*Larus ridibundus* L.); large numbers of such gulls died during the summer on fish ponds in eastern Bohemia where ducks were bred. They may have been the source of the ornithosis in the ducks (Strauss, 1956; Strauss *et al.*, 1957). Pursuing the spread of ornithosis by importation of infected ducks from Bohemia to eastern Slovakia, Hungary, and East Germany, the Czech investigators recognized that the annually recurring epizootics of duck ornithosis caused both large economic losses among the ducks and a considerable number of human infections, some fatal. At least 10,000 duck embryos were lost and nearly half of the 30,000 hatched ducklings died. Purchase and distribution of hatching eggs and breeding stock without veterinary control is blamed for this. Hospitalization, ambulatory treatment, and tedious prolonged convalescence seriously incapacitated the working force on the farms. Detailed study of one farm where the ducks were raised on 7 uncovered natural fish

TABLE 23.5

COMPLEMENT FIXATION REACTION AND ISOLATION OF ORNITHOSIS AGENT FROM DUCKS*

Indirect C. F. Titer	Number of Sera	Number of Isolations of Bedsonia
Negative	17	6
1:4	4	0
1:8	1	0
1:16	2	1
Prozone	1	1
Not tested	18	7
Total	43	15

* Data of Strauss et al., 1958.

ponds revealed that 1,350 of 7,000 ducklings (2 to 4 weeks old) had died in 2 months; during June at least 500 had perished. Clinical examination disclosed symptoms of ornithosis in 1950; from a sample of 43 ducks, 15 yielded a bedsonia. The relationship of the results of the indirect complement fixation reaction to the isolation attempts (Table 23.5) confirms that in acutely infected immature birds the antibody titers may be low and that the bedsonia may be isolated from ducks with no demonstrable antibodies in the peripheral blood.

Importation of 18,000 ducklings under unfavorable hygienic conditions and long distance transportation to poorly developed facilities resulted in 100 per cent mortality on one farm and 60 to 70 per cent on others (Strauss and Reistetter, 1960). In 6 poultry hatcheries 165,000 ducks were incubated; only 63,000 ducklings were hatched, and death or elimination due to poor growth reduced this number to 30,000 ducklings finally raised. Of 10 employees in close contact with the birds 8 contracted psittacosis.

Workers in the State Institute of Veterinary Science in the course of epidemiologic studies on ornithosis in eastern Slovakia from 1958 to 1960 observed that the infection appeared in seasonal outbreaks usually connected with importation of larger shipments of poultry for breeding. Epizootics in chickens were combined with outbreaks of pox, cholera, and coccidiosis (about 3 per cent). Disease in the ducklings was always accompanied by severe salmonellosis which caused losses up to 90 per cent. Breeding had to be discontinued. On farms under proper hygienic conditions, ample feed, and good care, losses were minimal.

In the investigations by Polony and his associates (1960), 314 avian specimens from 78 localities were examined; bedsoniae were isolated from 14 hens and chickens, 6 ducks, and 1 partridge. The highest incidence of infection, always along with salmonella (typhimurium, anatis, and enteritidis), occurred in ducks from May through July.

In 1958 and 1959 in similar outbreaks with heavy losses in the ducklings, but no illness in the adult ducks, 4 persons in Roumania handling the birds contracted ornithosis. The bedsonia isolated was pathogenic for mice and chick embryos but not for guinea pigs nor for pigeons by the intracerebral or intraperitoneal route (Popovici and May, 1960).

In 1955, members of families in the U.S.S.R. who had bought ducklings 2 days old contracted psittacosis (Krivinka, 1959).

The ornithosis agent has been demonstrated in 5 ducks in Roumania (Sarateanu et al., 1960).

Fritzsche and his colleagues (1956), describing the illness of a 41-year-old woman who worked as a plucker in a poultry processing plant in Germany, stated that adult ducks from Holland, having suffered as ducklings from "duck plague," were apparently responsible for the apparent and many inapparent (50 per cent) infections in the butchering and even office personnel.

The rapidly rising rate of psittacosis since 1957 in workers connected with duck fattening or poultry processing in the East German Republic initiated thorough epidemiologic studies. Surveys with the direct complement fixation test on 1,428 serum samples collected from ducks on 70 different farms disclosed 223 positive (>1:10), 100 suspicious (± 1:10), and 1,095 negative reactors. Thirty-five farms were considered infected, five others were placed in the suspicious class; 42 per cent of the infected farms were breeding farms; 57 per cent

were fattening establishments (Lehnert and Hille, 1960). Indirect complement fixation tests on serum samples of ducks delivered to a poultry processing plant W. in District D in Leipzig in East Germany showed that of 20 farms housing from 100 to 1,170 ducks, 10 farms were infected with ornithosis. Breeding and fattening establishments furnished from 5 to 50 latent infection reactors irrespective of the size of the flock or the general sanitation. No relationship between infection and management of the farms could be established, but introduction of ornithosis through infected eggs is suspected (Voigt et al., 1962).

Some observers working in East Germany (Bukowka et al., 1960; Siegmund, 1960) emphasized that ornithosis in ducks there has run a clinically inapparent course, that losses on the farms have been within expected limits (3 to 15 per cent) and that ducks as a source of infection have been recognized only after single or group human infection. In this respect the experiences in East Germany differ from those reported from Czechoslovakia, Roumania, Hungary, and elsewhere. Ortel (1960), in connection with his serologic and epidemiologic studies on an ornithosis epidemic among the employees of a poultry processing plant, reported that he could not isolate bedsonia from organs of ducks from regions originally considered infected because of the results of earlier indirect complement fixation tests conducted by the Veterinary Health Services. The ducks were processed in a poultry slaughterhouse in Halle. Bedsoniae were isolated on feathers and in excrements collected in the processing plant (Ortel, 1960). They were also found in the sputum and throat washings collected during the first few days of illness from 10 of 35 patients. It was difficult to maintain bedsonia of duck origin in mice after the first passage: none of the isolates produced fatal infections, but they did cause peritoneal lesions and microscopic particles in the cells of the peritoneal exudate (Ortel, personal communication). Evidently the bedsoniae studied were of low pathogenicity. In a report

Ortel (1961) summarizes the ornithosis situation in the DDR (German Democratic Republic). He lists the number of human infections due to contact with poultry, principally ducks, as follows: 1958, 23; 1959, 110; 1960, 683; 1961, 385; 1962, 160 with monthly peaks in August, September, and October.

In a systematic study of dead and sick ducks from a duck breeding and fattening installation (Illner, 1962b) bedsoniae were isolated as follows: in the second mouse passage from the enlarged spleen of an old duck hen that also had inflammation of the air sacs and oviduct; from 6 of 21 ducks 5 to 8 weeks old in either first (4), second (1), or third (1) mouse passage (salmonellosis and aspergillosis were also present); and from 5 of 15 visibly diseased ducklings (shortly after hatching to 14 days of age) in the first to third passage. The remaining 10 ducklings proved free from bedsonia yielded *Salmonella typhimurium* and *S. bredeney.* Young and old laughing gulls (*Larus ridibundus*) were also proven infected with bedsonia: 2 old birds in third mouse passage; pools of organs from several gull chicks (killed 4 to 6 days after hatching) in the first or second mouse passage, producing peritoneal exudate, splenic tumor, and liver necrosis.

Bedsoniae isolated from ducks and gulls were moderately pathogenic for mice, and despite suspicious gross lesions in older birds, the agent was more readily demonstrable in the organs of ducklings than of adult birds. The wild mallards and table ducks present on the farm did not yield bedsoniae. However, the pool of 10 eggs *from two separate nests* contained bedsoniae. Egg albumin, allantoic fluid or yolk sac material, embryos or liver of embryos removed under strict aseptic conditions from one gull egg without and one with embryo, and 2 pools prepared from uninculated duck eggs tested on mice produced serofibrinous peritonitis, enlarged spleen, and necrosis of the liver in mice of the second passage; microscopic findings were typical of those in an infection with a bedsonia of low virulence (Illner, 1962b).

In another study (Lehnert, 1962) 48 eggs from ornithosis-infected duck farms were examined. In one experiment the pool of embryos removed from 3 eggs incubated for 20 days caused bedsonia lesions in mice of the third and fourth passage. One of 9 ducklings killed at the time of hatch was proved infected. The isolates thus obtained when inoculated into the yolk sac of 6-day-old embryonated chicken eggs killed the embryos on the sixth to eighth days; large numbers of ornithosis particles were demonstrable in smears.

To ascertain whether in water fowl the bedsonia localizes in the ovaries and passes through the egg and thus maintains the infection requires more field and laboratory study. Some earlier experimental attempts to demonstrate transovarian passage in pigeons (D. J. Davis, 1935; Fritzsche et al., 1956) and turkeys (Davis et al., 1957a; Page and Bankowski, 1959) were unsuccessful.

In Czechoslovakia almost all 155 patients in the 1955 outbreak (Serý et al., 1957) kept ducks from the same hatchery. Strauss (1956) suspected that the incubator had been contaminated by eggs infected through transovarian passage.

Investigators of a poultry farm in Czechoslovakia where ornithosis and salmonellosis had prevailed for some time (Serý and Strauss, 1957; Strauss et al., 1957) discovered a concurrent destructive epizootic among young black-headed gulls in their nesting grounds in the vicinity of the duck farm. The organs of 31 of the 44 dead gulls harbored Salmonella typhimurium. Two of 15 young gulls yielded bedsonia in mouse inoculation tests. The observers called attention to the danger of salmonellosis and ornithosis being conveyed by gulls from their breeding places to poultry farms. They did not suggest the possibility that gulls feeding on dead ducks and frequenting the duck pens may have introduced the infection into the young gull population.

With confirmation of the high incidence of ornithosis and salmonellosis in gulls by East German workers, the ecologic relationship between ducks and other water fowl deserves further study. Ducks may be found on fish ponds simultaneously frequented by flocks of gulls. During the breeding season gulls in a wild bird reserve adjacent to two duck-breeding farms were found to be heavily infected with Salmonella typhimurium: 40 per cent of the examined birds and 5 per cent of their eggs; only 7 per cent of the ducks or other wild birds were proved infected. Salmonella was readily isolated from the ponds in the park, the duck ponds, the intestines of a carp, and three specimens of the common field mouse (Apodemus sylvaticus). These observations raise many questions about the role of wild birds in salmonellosis and possibly ornithosis in poultry breeding establishments (Koppel and Polony, 1958). The viability and persistence of bedsoniae shed in the droppings by wild and domiciliated water fowl into the pond water or elsewhere in the environment of the ducks are not known, nor are the principal causes of deaths among ducklings. The available limited data suggest that only a small percentage of deaths can be attributed with certainty to ornithosis; salmonellosis, hepatitis, pasteurellosis, and viral infections complicate the epizootiology.

Only a few bedsoniae isolated from ducks (in U.S.A., over 40; in Czechoslovakia, 33) have been studied to learn their microscopic appearance, antigenicity, and pathogenicity (Strauss, 1956; Strauss and Reistetter, 1960; Meyer and Eddie, 1956a). Compared with other members of the group, the isolates are of low virulence for white mice and embryonated eggs, but on adaptation may be fatal to embryos on days 3 and 5; the pathogenicity index (Page, 1959c) is between 3.5 and 4.8. They rarely produce fatal meningoencephalitis in pigeons or guinea pigs. Cross protection tests have not revealed differences between duck and pigeon isolates, but in toxin neutralization tests antiserum prepared with a duck isolate from California neutralized a duck isolate from Michigan but did not neutralize a pigeon strain toxin. As antigens, 5 isolates tested all gave group re-

actions. Antigens specific for duck isolates have not been demonstrated, and consequently their relationship to the isolates from gulls and their eggs cannot be evaluated.

Turkeys

Investigations that followed the outbreak of psittacosis in a poultry processing plant in 1948 inductively incriminated turkeys as the most likely source of the infection. The workers did not recall the processing of any unhealthy birds; in fact they said that the turkeys had been in an usually good condition (Irons et al., 1955). Three years later two additional human outbreaks, attention was focussed on a flock of turkeys with an illness undiagnosed for some time. Autopsy of two turkey hens showed cloudy thickened air sacs, brownish fluid in the peritoneal cavity and fibrinous pericarditis. Bedsonia was isolated from the pooled tissue suspensions from each bird (Bonev et al., 1952). This isolate was a highly toxic, elementary body agent with a broad host-infection spectrum. In toxin- and infection-neutralization tests it was distinct from isolates from psittacine birds and pigeons and from mammals; it is in some respects like the egret and Louisiana (Borg) pneumonitis bedsonia (Meyer and Eddie, 1953). Similarly toxic isolates have been found in flocks in New Jersey (Beaudette et al., 1956), Oregon, and Vancouver.

In the intervening 16 years, either isolations or serologic tests have revealed ornithosis in Arizona, California, Massachusetts, Michigan (Mack, 1955), Minnesota (Pomeroy et al., 1957), New Jersey a bedsonia of low virulence (Ilner, 1962b). (Beaudette et al., 1956), Ohio, Oregon (Osgood et al., 1956), Vancouver, British Columbia (Bowmer, 1958), in Alberta (Carlson et al., 1961), and New Mexico (Francis, 1960). Processing plant epidemics continued in Texas in 1951, 1952, and 1954 (Irons et al., 1955) and again in 1961 and 1963 (Rich, 1962; Rich et al., 1962; Peavy and Dickerson, 1963). Details on the

last two episodes in Texas, involving 11 employees, are not available.

During October, 1954, some turkeys in a flock of 2,100 in New Jersey had mild respiratory symptoms and diarrhea. After an illness lasting 5 to 6 days some birds died, and the total mortality reached 50 to 60. In November, inoculation of embryonated eggs with material from 4 dead birds yielded a highly virulent toxic isolate (Beaudette et al., 1956). Isolates from this flock were found at the Hooper Foundation to be indistinguishable from those from Texas and Oregon flocks. Poultry farmers and processing plant employees became infected through contact with this flock.

Attention was first called to the possibility of ornithosis in an Oregon flock in February, 1956, when the State Board of Health was notified by Veterans Hospital in Portland that a patient with symptoms suggestive of ornithosis had been exposed to turkeys (Osgood et al., 1956). This led to the discovery of 3 infected flocks on Sauvies Island near Portland, and in 2 (SK and J) of these flocks epizootics ran a particularly destructive course (mortality rate of 25 to 30 per cent). Because the infection in the flock was diagnosed during the epizootic, it was possible for the investigators to observe its course. Dead birds from the flock had been examined when illness was first observed in December. The clinical characteristics suggested erysipelas, but no evidence of this was found, and further diagnostic efforts were not productive (Dickinson et al., 1957). Birds continued to die and during the unusually wet winter the disease progressed. By the time the diagnosis was made the whole flock was heavily infected, many sick and dead birds could be seen, the premises were extensively soiled with droppings, and care of the flock had become a great burden. Treatment with tetracycline compounds in the feed improved the clinical situation but did not completely clear the organs of all birds of the bedsonia. Attempting to cope with such an outbreak in the absence

of the necessary detailed knowledge and policy is strenuous and disappointing to all concerned. In December, 1956, the infection reappeared in the breeding flock on one of the farms. Although it was treated, persistence of the agent in the tissues of the tested sample made it necessary to destroy the flock and compensate the owner, according to regulations enacted in the intervening months.

In December, 1957, the inspector in an Oregon plant condemned about a quarter of the hearts and livers of turkeys from another flock previously unsuspected of infection. A moderately virulent bedsonia was readily isolated from organs examined by a poultry laboratory. Periodic illnesses among the flocks were treated with medicated feed (terramycin, 200 gm/ton). In March, 1958, the poultry inspector removed the hearts and livers from 2 of 3 turkeys sent from the J ranch for processing. The lesions yielded a virulent bedsonia. Sera of a sample of turkeys reacted in dilution of 1:256. Chemotherapy for varying periods and doses of from 200 to 500 gm/ton were given until May when the entire breeding flock (1,189 hens and 63 toms) was processed. From 25 to 40 per cent of the hens and only 4 to 5 per cent of the toms showed lesions; 15 per cent of the toms had heart lesions. The agricultural authorities ordered destruction of the 113 turkeys remaining on the farm.

During 1959 the infection was again found on the J ranch. A virulent bedsonia was isolated from the viscera of culled and dead birds. In March, 2 sea gulls shot on the premises proved infected. A sample of the flock on a neighboring ranch (SK), previously suffering from ornithosis, showed 22 per cent reactors. Six of 146 tissue specimens collected during processing of turkeys from the J ranch were infected with a virulent bedsonia. By December, 26 per cent of 62 blood serum samples from turkeys of the same flock set aside for breeding proved positive. Visibly sick birds observed from January to April, 1960, reacted in the ICF test, and bedsonia was found in grossly

diseased tissues. Late in December, 1961, turkeys were inspected and sick birds were not seen on the farm, but when processed suspicious lesions were observed. In February, 1962, a bedsonia of low virulence was found in 3 lots of birds from J ranch; dead or sick turkeys had not been seen in the course of several inspections. In the ICF test of blood samples (32) taken in April, 1962, during processing of the flock 14 reactors were revealed (>1:32); in further blood samples collected in June the reactor rate was similar in 21 turkeys. The bedsonia from 4 of 7 tissues with lesions failed to infect mice by the intraperitoneal route and was fatal only by the intracerebral route in the dilution of 10^{-1} and 10^{-2}. The pathogenicity index determined with the second mouse passage was 4, indicative of low virulence.

The J turkey ranch was under observation for 6 years, and ornithosis of varying clinical severity reappeared annually in older poults and the breeding stock. The infection in epizootic form was invariably reflected in the direct and indirect complement fixation tests which showed that as high as half of the flock had been infected. Until 1961 a highly virulent bedsonia was in the gross lesions discovered when the birds passed over the processing lines. Supervision of the ranch was very difficult. When a shift from virulence to less virulence took place is not known. Poults though clinically well had visceral lesions in December, 1961, and a sample of serum collected while they were being processed showed a 40 per cent infection rate. Not until June, 1962, were organ specimens suitable for animal inoculation obtained. Apparently a bedsonia of low virulence evolved in poults growing up in 1961, and since the breeding stock was selected from the flock, the same agent was present when the adults were processed in 1962. For 6 years ornithosis tenaciously persisted on the J ranch. One would like to know the factors that maintained this niche, and in particular those responsible for the shift in virulence. The discovery

that pools of ground feather mites (*Glyco-phagidae*) removed from litter collected on the ranch after it had been depopulated of turkeys in 1959 carried a virulent bedsonia suggests that ectoparasites may in some way be involved in maintenance of the infection on turkey farms.

In 1958 a turkey ranch in New Mexico experienced a mortality of 300 birds in a flock of 2,200. The sick turkeys had diarrhea and cyanosis and pericardial and air sac lesions typical of those in ornithosis. A bedsonia was isolated by inoculation of allantoic cavity of embryonated eggs with suspensions of pericardial exudate. It was fatal to chick embryos on days 3 to 8. Tetracycline therapy (200 gm/ton of feed) reduced the mortality to 1 to 2 birds a day after the fifth day of treatment. Later the disease was completely arrested. No one contracted the illness from this flock (Francis, 1960).

In May, 1957, a poultry-processing plant outbreak in Canada led to isolation of a bedsonia from the only bird remaining on the farm in British Columbia from which the flock originated. The owner reported that the flock had been sick in February (Bowmer, 1958). Serologic tests (up to 25 per cent positive) conducted on five turkey flocks and isolation of a bedsonia *from sterile purulent pericardial exudate fatal to* mice on intracerebral (but not intraperitoneal) inoculation and from newly hatched turkey poults proved the existence of ornithosis in Alberta (Carlson *et al.,* 1961).

Ecology of turkey ornithosis had not been studied very thoroughly. With few exceptions the investigator rarely had the opportunity to collect data on the events that preceded the arrival of an infected flock of turkeys at a processing plant. Serologic and microbiologic examination made at the time the birds were processed or subsequently on the ranch where they had been raised proved that ornithosis due to bedsonia of high or low virulence was epizootic. When and how the infection was initiated and how long it had prevailed among the poults late in the year or among

the adult breeding flocks in spring and summer could not be learned. It is worth noting that 10- and 14-week-old poults were found infected in June (Davis and Delaplane, 1955). Since the droppings of acutely diseased birds yield the parasite in abundance, the feathers and the immediate environment heavily contaminated would certainly facilitate interbird exchange. Within a few weeks after the illness is first noticed, between 20 and 50 per cent of a turkey flock may reveal serologic evidence of infection. The natural mode of transmission has not been definitely determined. *Turkeys exposed to infective aerosols became acutely diseased, and 15 per cent died;* ingestion of a bedsonia produced inapparent infection followed by abundant shedding of bedsoniae. It was reasoned that ingestion is the most likely mode when the epizootic develops slowly; airborne spread by desiccated infected excreta is more likely when the disease spreads rapidly (Page, 1959a). In an experiment by Davis and Delaplane (1955) healthy poults were exposed to the air current collected from a pen in which artificially infected turkeys were held. None developed the disease, and the bedsonia could not be recovered from the tissues after an observation period of 5 weeks. In view of the observations made on pigeons, in all probability the turkey bedsonia may infect the host by the respiratory and the intestinal routes.

Spot serologic surveys in 1954 and 1955 in 11 states in which a complement fixation titer of 1:16 was considered indicative of present or past ornithosis showed that except in Iowa and Washington many flocks were infected. In 1957 a survey of 8 flocks ranging in turkey population from 350 to 1,761 birds indicated that 8 to 58 per cent (average 22 per cent) had antibodies to the psittacosis group antigen (Meyer and Eddie, 1957, unpublished data). Several thousand sera collected from turkey flocks in areas in Wisconsin and Minnesota where ornithosis had been proved yielded 18 per cent reactors with titers of 1:8 to above 1:128. Similar tests on flocks in different

parts of Minnesota yielded few low-titer reactors, but in 1957, 15 per cent had titers similar to those in 1954 (Pomeroy *et al.*, 1957).

Some similarities of turkey to pigeon ornithosis are striking. Many well established turkey-breeding and raising farms harbored a variable percentage of poults and adult breeding birds with latent infection. Man-made crowding and perhaps in some instances poor husbandry greatly favored exchange of the parasite. However, there is one difference: the infection was not contracted in the nest, the eggs were incubated and the poults were artificially brooded, many on the same contaminated premises where there were infected adult birds. Even if incubation and brooding are commercially done in special installations, it is reasonable to assume that many eggs originated from infected ranches. Contamination of the exterior of the eggs with bedsonia-containing excreta is suspected. Transovarian passage of bedsoniae to duck or sea gull eggs has been demonstrated, but the evidence in turkeys is more equivocal.

Several investigators searched for bedsoniae in dead turkey embryos or poults from eggs laid by breeding turkeys experimentally infected with highly virulent turkey bedsoniae. A total of 2,344 eggs were collected, incubated, and candled daily from the seventh day until hatching. The allantoic fluid from all dead turkey embryos was inoculated into 6- and 9-day-old chick embryos. Bedsonia could not be recovered. A portion of the hatched poults was placed on an isolated farm. There were no deaths due to ornithosis during a 12-week observation period (Davis and Delaplane, 1955). In another series of 2,954 eggs laid by breeding turkeys experimentally infected with the virulent JO ornithosis strain, although 500 of the eggs were laid during times at which the agent could be recovered from the blood of representative birds, bedsonia could not be found in the eggs. When fresh turkey eggs were inoculated with turkey bedsonia, the inoculated agent was not recoverable after

the ninth day after inoculation. The turkey bedsonia failed to live longer than 3 days on turkey eggshells or longer than 4 days on cotton swabs under egg incubator conditions (Davis *et al.*, 1957a). Attempts to isolate a bedsonia from 262 embryos of turkey eggs collected during an acute ornithosis epornitic were not successful; antibodies in titers as high as 1:64 and 1:512 were detected in the amniotic fluid or the serum of some turkeys (Page and Bankowski, 1959). During the extensive investigations of ornithosis in Oregon, 340 pipped turkey eggs and 110 dead turkey poults from flocks in which ornithosis was epizootic were, by repeated mouse passage tests, found free from bedsoniae. A sample of 200 eggs, hatched from eggs derived from infected hens and incubated on the infected premises, were tested before they were put on a range and proved sero-negative. In December an ornithosis epizootic with a fairly high progressive mortality rate broke out among a flock of 3,000 hens and toms that had been selected for breeding from the raised and previously tested poults. The sample to test the absence of infection in the poults may have been inadequate; since egg transmission could with confidence be excluded, other modes of infection into the flock were considered (Meyer and Eddie, 1957, unpublished). It was thought that on the open ranches turkeys might intermingle with a variety of avian and mammalian carriers and shedders of bedsoniae and might become infected. This received some support when sea gulls located on islands in Oregon, frequenting a farm where a turkey epizootic caused many deaths, were found to harbor bedsoniae indistinguishable from turkey isolates (Dickerson *et al.*, personal communication). Since gulls are avid scavengers it is impossible to say in which direction, if either, the infection passed. Both could contract it from a third source. In Texas several species of free flying birds of the *Ardeidae* family (mostly herons) were collected on a turkey ranch where ornithosis had been epizootic, but bedsoniae could not be found (Davis *et al.*, 1957b). Investi-

gation of an epizootic of ornithosis in Texas disclosed that the eye of Hurricane Carla had passed over the ranch in September and had deposited sea gulls singly or in small groups on the premises. Perhaps some of the turkeys became infected by water or sea birds and spreading of the infection took one and a half months to reach epidemic proportions (Rich, 1962).

Three of 15 pigeons, roosting on a turkey ranch for 1 year prior to an ornithosis outbreak in turkeys, furnished a bedsonia morphologically and by crude antigenic analysis and pathogenicity tests on mice and chick embryos indistinguishable from the one isolated from sick turkeys on the same premises. This suggested that the turkeys had acquired their infection from the pigeons. Adult pigeons heavily infected with a bedsonia of pigeon origin suspended in a wire cage from the ceiling of an enclosure holding 10 normal turkeys 17 weeks old apparently infected the turkeys. Within 6 weeks 9 of the 10 exposed turkeys developed indirect complement fixation titers ranging from 1:4 to 1:32. The infection was subclinical and transient, but there was serologic evidence that it was in turn transmitted to new groups of normal turkeys. No lesions were induced in the turkeys and no bedsoniae isolated (Page, 1960). The pigeon ornithosis agent used for these transmission studies was not described.

Sparrows are known to be hosts of bedsoniae. Without transmission experiments or specific antigen analyses, the isolation from sparrows of bedsoniae highly lethal for pigeons on intracerebral inoculation on a turkey ranch in central California does not yet clarify the ecology of epornitics.

Conclusive proof that ornithosis in turkeys on previously uncontaminated premises is an incidental event resulting from the intermingling with free-living avian or mammalian species carrying bedsoniae can only be proved by actual field experiments through carefully planned systematic environmental studies over many years on a number of turkey flocks established and maintained in different geographic areas. Whether such experiments are necessary will depend largely on whether the results of extensive serologic surveys widen and confirm the prevailing view that inapparent ornithosis in turkeys is more universal than generally recognized or admitted. Explanations of the origin of epizootics, recognized since 1948 on account of their relation to occupational human infections or because the poultry inspectors condemned diseased birds, have largely depended on verbal reports by the grower. Turkey raisers have commonly said that they have never seen this form of illness or abnormal death before and that it must have been introduced. But "how" neither he nor the investigator has been able to establish. According to available records, only 2 ranches, on which ornithosis was proved, have been under observation in subsequent raising and breeding seasons after the catastrophic epizootics. On one ranch 83.5 per cent of 289 sera were positive; the bedsonia was of low virulence; the following year 161 sera collected during processing of part of the flock gave no indirect complement fixation reactions. Other follow-up studies could not be made. By contrast, annual epizootics of varying severity plagued another breeder and grower for at least six years.

Feather mites belonging to the glycophagidae contaminated with bedsoniae have been found in the bedding on the premises many months after depopulation of living birds. It is suspected that these arthropods may assist in the maintenance of the disease agent through generations of turkeys (Eddie et al., 1962).

A variable percentage of poults may acquire the infection without showing symptoms. As they grow older and during the breeding season, for reasons entirely unknown, the ornithosis agent may undergo changes in virulence in certain flocks. More birds become infected and shed; some show symptoms and die. If highly virulent mutants appear, the epizootics become economically serious and a source of disease both to the raiser and his workers and to

the employees in processing plants exposed to grossly diseased turkeys. The ecology is quite similar to that of pigeon or duck ornithosis; it is rarely man-made. Wild turkeys serologically and microbiologically examined in Utah and Ohio were not infected.

Ornithosis in domestic turkeys has been recognized during the past 15 years; it has principally affected breeders' flocks. It became the subject of study when the parasite infected large human occupational groups. Through direct isolation of bedsoniae from the viscera of apparently normal turkeys, or indirectly through serologic surveys, the wide geographic distribution of ornithosis in turkeys in the U.S.A. has been established. Strangely, these facts are either overlooked, deliberately suppressed in order to protect the poultry industry, or despite overwhelming evidence the specificity or validity of serum tests is questioned. The number of farms raising turkeys dropped from 169,807 in 1954 to 86,712 in 1959; the number of turkeys produced increased from 67,693,000 to 84,493,000. In both Texas and Oregon the number of farms raising turkeys has dropped; Texas, 1954, 25,356; 1959, 11,295, and Oregon, 2,386 to 994. Examination of even a small percentage of these farms presents technical difficulties, but it must be re-emphasized that for example in Oregon, random chosen samples of 50 sera from nine farms yielded from every one at least 1 to 12 sera which reacted in dilutions of 1:16. Since the infection was low grade, the mortality not abnormal, and the marketability not affected, neither the poultry industry nor the official livestock control agencies could be interested in extending the surveys to secure a broader understanding of the ecology of ornithosis in turkeys.

Chickens

Millions of chickens are raised and marketed annually in the United States, but an epizootic or epidemic of psittacosis in chicken processing plants has not been reported. Sporadic cases have been attributed to contact with chickens (Karrer et al.,

1950a; Meyer, 1952; Irons et al., 1955; Scruggs, 1957), and there is extensive serologic evidence of ornithosis in chickens. In indirect complement fixation tests of 464 chicken sera from hatcheries in California, Iowa, Michigan, Texas, and Washington, 18.3 per cent were positive (Karrer et al., 1950c; Eddie and Meyer, unpublished data). In Connecticut, of 319 serum samples, 31 were positive to some degree (12, 1:2; 12, 1:4; 6, 1:8; 1, 1:16), the reactors were confined to 4 flocks and the birds were, except in 1 flock, over 6 months old (Rindge et al., 1959). Limited attempts to isolate a bedsonia from chickens in the United States have been successful in a few instances. Pools of emulsified spleen, liver, and kidneys of 4 of 35 chickens from a farm in New Jersey yielded a bedsonia of low virulence for mice but fatal to parakeets, ricebirds, and pigeons, on intramuscular and intracerebral inoculation (Meyer and Eddie, 1942). An isolate of low virulence was obtained from the organs of 2 emaciated chickens from a flock in California (Karrer et al., 1950a). One of 6 hearts with extensive bacteriologically sterile fibrinous exudates from chicken fryers in a processing plant in Oregon showed ornithosis particles and on mouse passage yielded a bedsonia that infected but was not fatal to 1-month-old chicks. It persisted in 3 of 4 chicks for 1 month, but healthy chicks placed in the same enclosure did not contract the infection. One serum specimen of 32 collected from fryers of this flock reacted in a dilution of 1:32. An inapparent epizootic was revealed in Oregon in the course of surveys: 5 of 7 specimens reacted (2, 1:2; 1, 1:4; 1, 1:16; 1, 1:32).

According to recent reports from Hungary, ornithosis diagnosed by isolation of bedsonia from hens and chickens has been an epizootic fatal disease causing considerable economic loss (Derzsy, 1958). Polony and his associates (1960) in their observations on ornithosis in eastern Slovakia in 1958 and 1959 recovered 14 isolates of low virulence from dead or sick chickens (1 to 2 years old) and chicks (2 months old); salmonella was also found in these birds.

Simultaneous outbreaks of pox and fatal cholera caused losses of from 5 to 15 per cent.

Bedsoniae were isolated from a group of free-living chelated mites from White Rock roosters in the Midwest that for many years had furnished birds invariably free from ornithosis (Eddie *et al.*, 1962). This was the first isolation of a bedsonia from an insect *(Menopon gallinae* L.). Interpretation of this finding is at present impossible. It is being ascertained whether this ectoparasite could introduce ornithosis into a flock and whether it could perpetuate or spread it.

Domesticated Pheasants

Inductive epidemiology of human infections has pointed to commercially raised pheasants. The owner of a large pheasant farm in Illinois contracted pneumonitis and his serum gave a positive complement fixation reaction (1:128). Several pheasant sera have been positive in the complement fixation inhibition test in significant titers (1:64) (Morrissey and Meyer, 1951). In 1956, through the courtesy of Dr. Richard E. Shope, spleens were obtained from 3 pheasants from a game bird farm. A bedsonia of low virulence was isolated from one. In pathogenicity tests on mice it has behaved liked a pigeon isolate. Fifty-four pheasants of a different lot from the same farm were sero-negative in both the direct and indirect tests.

CLINICAL FINDINGS AND PATHOLOGY

Pigeons

Coles (1940) was the first to describe ornithosis of pigeons. The birds were visibly sick, listless, and showed no interest in food; vent feathers were soiled with liquid feces. At necropsy the lungs were edematous, the liver and spleen swollen. There were moderate aerocystitis in the abdominal air sac and moderate catarrh of the intestines. There were elementary bodies in the blood, lungs, and spleen, and a bedsonia was isolated in mice. *Haemoproteus columbae, Trichomonas hepatica,* and *Sal-*
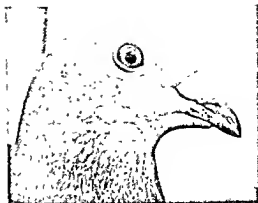
FIG. 23.2 — Ornithosis in racing pigeons. Normal eye at top; moderate conjunctivitis, center; severe conjunctivitis, bottom. (Courtesy of Professor Jac. Jansen, Utrecht, Netherlands.)

monella typhimurium were also found in this flock.

The clinical picture is never typical. Infected squabs are usually undersized and feeble. Diarrhea is common, and vent feathers are matted with grayish-green concretions. Adults that have shown no premonitory symptoms may die suddenly. Others are weak, emaciated, lose their appetites and have diarrhea. The following has been considered typical of the course in acutely diseased racing pigeons (Jansen, 1955, 1959; van Vloten, 1954): unilateral or bilateral serous conjunctivitis, rattling respiratory noises, serous purulent rhinitis ("dirty nostrils"), incapacity in flight, bluish discoloration of the skin over the pectoral muscles, anorexia, and soft feces. Fritzsche and his associates (1956) described respiratory distress with creaking rattling sounds, temporary paresis of the limbs and neck, edema of the vent, and severe diarrhea. According to both Coles and Jansen, ornithosis should be suspected whenever a pigeon is affected with conjunctivitis (Fig. 23.2). The ornithosis agent can be readily isolated by intracranial inoculation of mice with exudate or swabbings from the eye (Monreal, 1958). Experimental studies indicate that acute or latent ornithosis may be produced in pigeons. Carrier and racing pigeons exhibit respiratory disturbances, serous nasal discharge, turbidity of the eyes, swelling of the lids, and conjunctiva combined with pronounced exudates leading to matting of the feathers around the head. Anorexia leads to weakness followed by roughened feathers and quiet sitting in the corner of the pen or cage.

Adult pigeons may recover within a few weeks. In squabs or squeakers the disease is more severe and the mortality may be high. Recovery is incomplete in many birds, growth is retarded or impaired and the pigeon breeders are obligated to eliminate the birds from the flock. Adult pigeons may remain shedders for months. Fancy and poultry pigeons held in lofts exhibit principally intestinal symptoms and reduced fertility (Meyer, 1942; Fritzsche *et al.*, 1956; Weyer and Lippelt, 1956). Transient paralysis has been observed in the U.S.A. and Europe (Jansen, 1959).

At necropsy two types of lesions are observed (Meyer *et al.*, 1942a; Hughes, 1947; D. J. Davis, 1955; Fritzsche *et al.*, 1956; Monreal, 1958). In young birds a dry fibrinous plastic exudate covers the air sacs and the acutely inflamed serous membranes of the pericardium, liver, and intestinal coils. The liver is swollen, hemorrhagic, occasionally mottled, and saffron colored; the moderately enlarged spleen is dark pink or purple and very soft. Parenchymatous changes in the kidneys are common. If there had been catarrhal enteritis, urates may have accumulated in the cloaca. In less acute infections the inflammatory exudate may be limited to the lining of the abdominal air sacs.

In adult birds with relapsing latent infection, the enlarged liver with intense congestion and rounded edges is striking. Necrotic foci the size of pinheads are scattered throughout the liver of some pigeons. The spleen may be quite enlarged; in a few cases subcapsular hemorrhage is followed by rupture of the capsule, bleeding into the abdominal cavity, and sudden death. Enlargement of the spleen does not seem to be so constant in pigeons as in psittacine birds (Fig. 23.3).

In sero-positive adult birds from which a bedsonia has been isolated, there may be no gross anatomical lesions, and the size of the spleen may be in the normal range (3 x 11 mm.). Occasionally, normal sero-negative pigeons from which a bedsonia has not been isolated may have large spleens (7 x 18 mm.) (Weyer and Lippelt, 1956). In infected racing pigeons enlargement of the spleen, discolored liver, swelling of the kidneys, and grayish discoloration and enlargement of the pancreas with pinhead necrotic lesions have been described (Fritzsche *et al.*, 1956). The pancreatic lesions are not due to the ornithosis agent; they consist in faultily stained cells containing elementary bodylike elements surrounding the Langerhans cells and in-

FIG. 23.3 — (A) Spleens of pigeons proved to be infected with ornithosis virus. Natural size. (B) Two spleens and one liver of mice infected with psittacosis virus. Liver is necrotic. Small spleen not infected. Natural size.
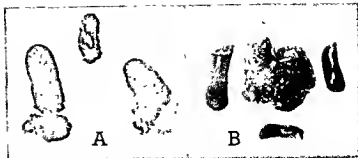
clusions caused by the intranuclear inclusion virus (Smadel et al., 1945). Both the ornithosis and intranuclear inclusion agents can cause epizootics in pigeons.

In a few pigeons with caseous pneumonic foci, Salmonella typhimurium (var. Storrs) has been isolated from the cheesy material. In such cases the pulmonary lesions are probably not part of the ornithosis. Organs and exudates from pigeons with pure ornithosis are sterile when planted on ordinary medium or medium enriched with brilliant green broth. Smears prepared from the fibrinopurulent exudate in the pericardium or over the liver are usually rich in particles, either free or in the cytoplasm of monocytes. Sections of exudates on serous surfaces, such as the pericardium, reveal intense inflammatory infiltration with large mononuclear cells and lymphocytes and few polymorphonuclear leukocytes. The reaction may penetrate into the superficial layers of the myocardium. Vascular congestion in the liver and kidneys is associated with cloudy swelling and varying degrees of necrosis. Hemorrhagic areas are present in the spleen.

Pigeons, like parakeets, ducks, and turkeys, with or without complement fixing antibodies, may harbor the bedsonia. Most pigeons from infected commercial pigeon lofts are immune to intramuscular injection with pigeon isolates, but they contract fatal meningoencephalitis when the injection is made intracranially. The remarkable resistance of this species to ornithosis bedsonia by feeding or by intramuscular injection is probably attributable to acquired immunity, in many instances conditioned by persistence of the bedsonia in

the tissues. According to Jansen (1955), there is no evidence that a pigeon that has recovered from ornithosis has contracted it a second time. Ornithosis in pigeons is very contagious, so it is likely to affect all racing pigeons kept by one owner, but, despite the high morbidity, the mortality may be very low. Recovery may be rapid, with a few birds being completely normal a month after the onset of symptoms, but the illness usually lasts 2 to even 4 months. The Pigeon Health Service in the Netherlands does not recommend the separation of infected birds because "it is much better and less disheartening when all the birds are sick together, instead of having a small number of patients among his pigeons at regular intervals."

Chemotherapy with streptomycin in two subcutaneous injections (0.5 ml. saline containing 25 mg. of streptomycin and 25 mg. of dihydrostreptomycin sulfate) three days apart has induced rapid clinical improvement (Jansen, 1955; van Vloten, 1959). Dekking (1961) pointed out that the ornithosis agent is not susceptible to streptomycin and recommended the use of tetracycline as originally tried by Meyer and Eddie (1955). Prolonged oral treatment, consisting of offering the pigeon a mash containing 0.5 mg. of chlortetracycline per gm. of feed for 1 month, has eradicated the bedsonia from pigeon tissue. Contrary to the warnings of Fritzsche and his associates (1956), who observed shock following oral therapy with chloramphenicol (700 mg/ bird) for 1 week, side effects or death were not seen after oral treatment with tetracycline.

Chickens

Little is known about the infection in this bird. It has almost always been subacute or inapparent. Nothing is known about the clinical disease, and at necropsy, aside from enlarged liver and spleen, the air sacs appear normal. In very rare instances, and then only in fatal infections in young birds, fibrinous pericarditis has been present. Attempts to produce these lesions experimentally by infecting chicks intratracheally with chicken or pigeon isolates have failed. The birds remained clinically well but shed the bedsonia for several days, and in 3 months the indirect complement fixation titers rose. By that time the agent could not be found in the viscera. A German textbook on diseases of poultry (Fritzsche and Gerreits, 1959) mentions (on p. 112) only the clinical and pathologic observations in the U.S.A.

Ducks

Ornithosis observed in ducks in the United States has been clinically inapparent (Meyer and Eddie, 1952; Korns, 1955). Clinically normal ducklings and adult ducks have had no gross anatomical lesions, despite the presence of bedsoniae in the spleen, liver, and kidneys and intestinal tract of about a third of the birds examined. Concurrent and secondary infections are quite common, but little is known about their causal role in epizootics on duck farms. Diagnostically useful clinical symptoms suggesting ornithosis in ducks have been singularly absent.

An entirely different picture appears in the excellent Czechoslovakian investigations, in particular those by Serý, Strauss, and their associates. While studying 3 small foci in a district in central Bohemia, where 600 of 2,000 ducklings died, Kukásek and Strauss (1956) noted stunted growth, weakness, unbalanced gait, watery diarrhea, and cachexia. During an epizootic in east Bohemia, of 3,670 ducks several weeks old derived from the same incubator on an infected farm and kept by small farmers, about a fourth in the acute stage showed bilateral purulent conjunctivitis and keratoconjunctivitis, which may lead to blindness (Fig. 23.4A). Serous or purulent yellowish discharges were present even at the auricular and nasal orifices; around the eyes the secretion formed a narrow strip. In the more advanced stages the feathers around the eyes were glued together and covered by crusts of dried secretions. In several instances the bedsonia was isolated from diseased ducks (Serý et al., 1957). The photographs furnished by Dr. V. Serý impressively illustrate the ocular lesions in epizootic ornithosis. The swollen lids nearly always close the eye (Fig. 23.4B).

A prominent symptom in all acute infections is greenish or gray-greenish droppings that contain bedsoniae but not salmonella. The agent is massively excreted onto the feathers, and this endangers the health of the pickers.

As the disease progresses, emaciation and atrophy of the muscles become marked.

When the clinical phase has completely disappeared, serologic and isolation tests in 8-month-old birds proved them to be carriers and shedders (Trojan and Strauss, 1955). However, only ducklings up to the age of 4 months were sources of human infections. When the epizootic had subsided, although ducklings showed no gross evidence of the infection, latent ornithosis was readily demonstrable. When these latently infected birds were then held under unhygienic conditions, the disease became manifest and in some instances infectious for man.

During the epizootics in Slovakia, though conjunctivitis was recognized in only 3 to 25 per cent of ducks, the sera of from 10 to 80 per cent reacted in the indirect complement fixation test in a dilution of 1:4 or higher. More visibly diseased ducks were observed in flocks held in an unsanitary environment.

Postmortem findings in infected ducks are similar to those in pigeons. In ducklings a few weeks old, acute or subacute inflammation with exudation on the serous membranes of the pericardium and air

FIG. 23.4A — Sera-purulent discharge and crusts from eyes, auricular and nasal orifices of ducks suffering from ornithosis

FIG. 23.4B — Bilateral conjunctivitis and keratoconjunctivitis. Swollen lids closing eyes in ducks with ornithosis. (Courtesy of Dr. Vladimir Sery, Prague, Czechoslovakia.)

sacs accompanies congestion of the liver and spleen. Hepatic necrosis may affect only small foci, or it may extend to large, marginal, yellowish areas; the latter are usually characteristic of concomitant salmonellosis. The serofibrinous pericarditis is well illustrated by a photograph kindly furnished by Dr. V. Serý (Fig. 23.5).

Latent infection of older birds is indicated by hepatomegaly and pronounced splenomegaly (spleen may measure 3.8 x 1.2 to 2.2 cm). Aside from inflammatory areas in the lungs, older ducks present saffron-colored livers, splenic tumors, and variable pericardial and air sac inflammatory reactions (Krivinka, 1959). Microscopic examination of organs of 8 ducks from the farm that supplied the poultry responsible for six occupational infections in Austria revealed unusual findings: the architecture of the liver was greatly transformed by necrotic, fully degenerated parenchymatous elements; new formations in the bile duct interspersed by inflammatory infiltrations convey the impression that these changes developed because of an acute liver atrophy. In the Giemsa-stained section the Kupffer cells contained small and large bine particles that could be identified as bedsonia because the larger granules consisted of phagocytized nuclear detritus of erythrocytes as a sequel to intravascular hemolysis. Throughout the voluminous tumor-shaped splenic tissues nodular infiltrates of necrotic cells were distinct; the myocardium showed fatty disorganization of the fibers (Furst et al., 1957).

On a duck breeding and fattening farm in Germany where within 21 days 500 ducklings had died, the management reported that paralysis and spasm preceded death by a few days. Systematic postmortem examinations of dead or moribund ducklings and adult ducks over a period of 18

FIG. 23.5 — Serofibrinous pericarditis in duck yielding bedsonia. (Courtesy of Dr. Vladimir Sery, Prague, Czechoslovakia.)

months revealed pericarditis, pneumonia, enlarged spleens and livers, inflammation of the abdominal air sacs and oviducts (17 of 19 birds examined), and oophoritis. Bedsoniae were seen in stained smears prepared from the pericardial exudate of ducklings under 2 weeks old and of young ducks 5 to 8 weeks old that had similar grossly observable lesions. Since salmonellosis and aspergillosis were encountered and bedsonia was isolated by mouse passage only in 12 of 71 examined ducks, it is reasonable to suspect that other infectious agents may have caused the gross lesions. But 5 ducklings 3 to 4 days old, from which a bedsonia of low virulence was isolated and that were bacteriologically free from salmonella, had saffron-yellow livers and patches of pneumonia (Illner, 1962b). In ducks less than 6 days old, intravenous injection of suspensions prepared from infected spleens has caused a typical disease and gross changes with great regularity. All livers and spleens were enlarged due to

numerical increase of the reticuloendothelial cells; fibrinous exudates were present in the pericardial sac and in the air sacs. Bedsonia particles were found in all air sac membranes stained with the Macchiavello stain (Jacobs, 1957).

Geese

Czechoslovakian investigators (Trojan and Strauss, 1955; Strauss et al., 1960) incidental to their studies in east Bohemia and Slovakia isolated a bedsonia from geese. The clinical and autopsy findings have been similar to those in ducks (Sery, 1962) (Fig. 23.6). Additional isolations are on record from Roumania (Sarateanu et al., 1960) and the U.S.S.R. (Terskikh et al., 1961).

Turkeys

Two flocks in Oregon furnish information on the clinical course of epizootic turkey ornithosis (Osgood et al., 1956). The date of onset of the first illnesses is not

FIG. 23.6 — Microscopic lesions of hepatomegaly. Lesion yielding bedsonia observed in goose suffering from subacute ornithosis. (Courtesy of Dr. Vladimir Sery, Prague, Czechoslovakia.)
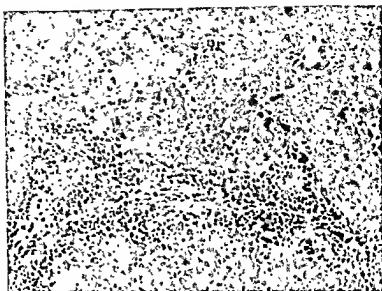
known, but in December some minor losses (about 2 deaths a week) were attributed to fowl cholera or erysipelas; laboratory tests did not confirm this suspicion. Of 1,796 turkeys processed, 79 were condemned because they showed "air sac disease." The losses in the remaining birds increased in January, and by March the daily mortality reached 50 to 60 birds. Total losses were 2,000 (30 per cent) of one flock and 350 (20 per cent) of another flock.

There were dead turkeys in the pens and yards, and the balance of the flock in general was droopy, anorexic, and feverish. Many were weak and reluctant to move even when disturbed. On being driven some staggered and fell, manifested acute respiratory distress, and died within a few minutes. Some were extremely emaciated, their wattles dry and cyanotic, and their eyes dull and sunken. Many had eye lesions ranging from slight inflammation of the conjunctiva to complete necrotic obliteration of the orbit. The grounds of the pens were heavily blotched with soft or liquid yellowish droppings, some of which were blood tinged. Soiling and matting of the vent feathers were common, and the cloacas of many birds were everted. Some birds had lost many feathers from the

breast and back, exposing large areas of flesh. The keels of many birds were ulcerated and abscessed, and the wing tips were heavily contused and abraded by the efforts of the birds to rise to their feet. Evidence of cannibalism was noted on the carcasses of dead birds and quite frequently on the moribund and sicker ones.

Egg production was far below normal in both flocks. Both hens and toms were infected, but in one flock the infection was slower to manifest itself in the toms, was not as severe, and the mortality was lower. Such differences were not as conspicuous in the other flock.

Necropsy of several sacrificed birds that had been ill and several birds that died presented the following lesions: Inflammation of the serosal surfaces with fibrinous exudation was consistently observed. Air sacs were inflamed and thickened and in one or two instances contained masses of cheesy exudate. The mesenteric and serosal surfaces of the intestine also were injected and inflamed in many birds. The abdominal cavity of a few birds contained fluid and fibrin deposits. The livers were slightly enlarged and off color, and in many birds the surface was covered with whitish, fibrinous films. These films ranged

FIG. 23.7—Ornithosis in turkey. Note fibrinous pericarditis and enlargement of the liver.

from small to extensive patches covering the surfaces. Few spleens were enlarged. The pericardium of almost all birds showed evidence of inflammation, with fibrinous exudation, and epicardial adhesions were beginning to form (Fig. 23.7). The pericardial cavity of some contained transudate and fibrinous flakes. Myocarditis was evident in large, soft, flabby hearts, and in some cases the coronary vessels were injected. Diffuse pneumonia was seen, but pulmonary edema was more often noticed. Many birds were markedly cachectic and poorly feathered and had decubital breast ulcers. Yellowish-green soiling of the vent feathers was a consistent finding.

At processing, about 5 weeks after the acute epizootics were arrested by the use of tetracycline compounds, the residual lesions in a large part of both flocks were critically appraised. The lesions were classed in three groups: (A) No lesions, or only slight, well healed residua—slightly thickened pericardium or small (less than 1 cm. in diameter) fibrinous to fibrotic patches on the epicardium or liver. (B) More extensive, but not active, lesions—fibrinous to fibrotic patches on the liver, chronic pericarditis with or without epicardial adhesions. (C) Severe lesions in an active stage or in an early stage of resolution—chronic pericarditis with fibrinous exudation on the pericardium, liver, air sacs and peritoneum, or extensive chronic lesions with massive adhesions and marked cachexia.

Of 1,200 hens of one flock processed, 59 were condemned. All birds with Group C lesions and other birds with diseases unrelated to ornithosis were condemned. The Agricultural Marketing Service inspecting veterinarian condemned at least 80 per cent of the livers and 90 per cent of the hearts. Lesions in 1,081 (85 per cent) were grouped as follows: 925 (85 per cent) Group A; 120 (12.5 per cent) Group B; 26 (2.5 per cent) Group C. The lesions in another infected flock of 1,808 were similar: 86.6 per cent Group A; 11.1 per cent Group B; 2.2 per cent Group C. From flock 1 there were 695 that had as high a rate of lesions, although less severe. This supported the owner's report that the mortality was less in the toms and the clinical course less severe.

The findings in flock 2 were essentially the same. More than 90 per cent of the birds showed some residual lesions on the heart and liver. Most had no more than small fibrino-fibrotic patches on the epicardium and slight adhesions to the pericardium. This was true in both flocks. In ordinary inspection many of these lesions would have been overlooked, but these were particularly well observed because of the foregoing infection.

The pathologic changes in the gonads in both toms and hens were strikingly different in the two flocks. In flock 1 reproductive functions were interfered with during the epizootic but improved after treatment. Neither egg production nor fertility was improved in flock 2 in spite of marked

clinical improvement after similar treatment. In flock 1 more than 90 per cent of the hens showed evidence of prolific ovulation. This was apparent even when there were advanced lesions in other organs. There was very little pathologic change in the ovaries or testes. In flock 2 more than 60 per cent of the hens had nonfunctioning or diseased ovaries. Most were simply atrophic or blighted, but others were necrotic, hemorrhagic, caseated, or inspissated masses. In a few instances, fully shelled eggs were incarcerated or adhered within the oviduct. These apparently were obstructed by rapidly forming fibrinous adhesions that later fibrosed. Gonadal lesions were also found in toms in flock 2. Atrophied testes were common; many were greenish. Well inspissated old hemorrhagic masses enveloped several testes. Adhesions to surrounding structures were also common. Flock 2 was much further advanced in egg production when the epizootic reached its peak than was flock 3. Also, flock 2 received high levels of sulfonamides over a longer period. Either or both of these may have been factors.

Several enormously enlarged spleens were observed in flock 1, but most of the spleens were of normal size in both flocks.

Published descriptions by other observers on naturally and experimentally infected flocks differ in a few details. They all stress that infected turkeys may look normal at antemortem inspection and still have typical lesions at autopsy (Carlson et al., 1961; Francis, 1960).

Early observations, clinical and pathologic, in Texas were made on turkeys of mature breeding stock in the latter part of the laying season. However birds 10 to 14 weeks old were also involved in the outbreak. Birds of all ages were susceptible but the mortality was much higher in younger birds. Among the symptoms noted were yellowish-green diarrheal droppings, droopiness, coughing, rattling, or other signs of respiratory involvement. The most prominent lesions of turkey ornithosis may be confused with those of other diseases characterized by fibrinopurulent inflammation of the serous surfaces of the thoracic and abdominal viscera. Frequently the only organs visibly affected are the spleen, liver, and kidneys (Boney et al., 1952; Pate et al., 1954). In an outbreak in New Jersey (Beaudette et al., 1956), aside from mild respiratory symptoms, the most striking symptom was diarrhea in which the droppings were sulfur yellow and of an unusually disagreeable odor. Turkeys that died did so after an illness of 5 to 6 days; rigor mortis was complete within a half hour after death. Autopsy revealed necrosis of the liver and spleen, congestion of the lungs, and hemorrhages in the skeletal muscles. The air sacs showed evidence of infection in absence of sinusitis.

Experimental ornithosis has been studied to find pathognomonic lesions to differentiate turkey ornithosis from other poultry diseases (Davis and Delaplane, 1958a). Turkeys have been readily infected with a Texas isolate by the intracranial, intratracheal, intraperitoneal, or intra-air sac route. Poults and adults were infected by the intratracheal route with allantoic fluid collected from chick embryos dead as a result of a highly virulent turkey isolate. The incubation time ranged from 4 to 7 days depending on the age of the turkeys. Mortality was influenced by the same factor: within 7 days it was 100 per cent in poults 1 week old; it was 25 per cent in birds 6 weeks old. In the age group of 3 to 6 months the average mortality was 22 per cent.

Pericarditis was the most constant autopsy finding. The spleen was rarely enlarged, but the liver was streaked with gray or green bands. Biliary stasis was indicated by greatly enlarged gallbladder, and the intestines contained bright green feces throughout the entire length. Aside from the fibrinous inflammation of the pericardium, the incidence of exudates over the liver (perihepatitis) was more marked in the older birds and was apparently influenced by the duration of the illness. The incidence of congestion and edema of the

lung was high in poults, but was usually absent in older birds (Boney et al., 1952; Pate et al., 1951; D. E. Davis, 1955).

A different experimental approach was chosen by Page (1959a) to study the pathway of infection and pathogenesis of an ornithosis isolate from a turkey involved in an epizootic and human occupational infection. Broad-breasted Bronze turkey poults 10 to 23 weeks old proved free from ornithosis were exposed to infective aerosols. They became acutely ill, and about 15 per cent died. Infected birds were sacrificed at various stages of the disease in order to learn the extent of multiplication of the bedsonia and the pathway of infection. Within 24 hours and until death high concentrations of the inhaled bedsonia were found within the lung. Bedsonia were found in the blood within 18 hours and were thereby distributed to tissues throughout the viscera. The epi- and pericardial surfaces and peritoneal serosa became infected by extension from the air sacs. Continued multiplication in these sites intensified the inflammatory response in the form of heavy deposits of fibrinous exudates.

Recovery from the acute experimental infection was followed by complete disappearance of the agent by the seventieth day, demonstrable by mouse inoculation of small fragments of liver, spleen, and kidney. However in a few instances bedsonia were found in the pericardial sac and kidneys.

In this connection, systematic field observations were made to determine the extent of latent infections in well turkeys. Some spleens removed at the processing lines were tested singly by inoculation of mice; of 98 selected from 215 normal or moderately enlarged spleens from 3 different flocks, 7 yielded isolates. Only 4 of these 7 birds had been sero-reactors.

Questions remain about the pathogenesis. Ornithosis has at times been a highly contagious disease, but limited experimentation by blowing the exhaust air from an enclosure holding infected sick turkeys into an adjacent pen with healthy turkeys failed to infect the birds (Davis and Delaplane, 1958b). Artificial spray aerosols readily infected; simple feeding with a pipette into the crop of the bedsonia in the dose of 310,000 mouse LD_{50} produced no clinical effects or serologic response. By inserting gelatine capsules containing 40,000 mouse LD_{50} of the virulent New Jersey isolate into the esophagus, a low-grade infection was established in a few turkeys within 2 weeks. The ingested agent multiplied, invaded the blood stream, was excreted by some birds, and caused severe disease in healthy turkeys exposed in the same pen. By the end of 2 months all the fed and contact birds had rising indirect complement fixation titers, gross lesions, and bedsonemia.

If ornithosis were initiated in a few turkeys by inhalation or ingestion of infective material of extrinsic origin, it could permeate rapidly through a flock, particularly if the birds were crowded and infected excreta were to be either ingested or inhaled (Page, 1959a); the spread may in some way be promoted by cannibalism.

Transmission experiments with isolates of low virulence administered by various routes successfully reproduced clinical and anatomical ornithosis, with a case mortality rate of up to 42 per cent in 1-day-old, 2 per cent in 10-day-old, and no death in 14-day-old turkey poults. Thirty (in groups of 10) healthy control turkeys 6 weeks old, held in the pen with the 65 diseased experimental birds did not get sick, but recovery of the bedsonia and appearance of antibodies proved that isolates of low virulence are as contagious as virulent ones (Gale, 1960). Even isolates from pigeons transmitted to turkeys by exposure to diseased turkeys, apparently by the aerogenic route, were contagious in contact tests (Page, 1960). The isolates of low virulence induced no clinical signs of disease except for fever and marked leukocytosis in turkeys over 2 weeks old, even though internal lesions were extensive.

The localization of the inflammatory

lesions differed in no way from those observed in turkeys infected with highly virulent bedsoniae. Autosterilization has been apparently less active: the parasite of low virulence seems to be better adapted to persist in the organs since it has been found there for over 120 days. The highly virulent parasite disappears around the seventieth day (Gale, 1960). The cellular proliferation, fibrosis, necrosis, and capillary hyperplasia were nearly identical with those noted on specimens collected in an active epizootic among turkeys in a flock parasitized by highly virulent agents (Gale et al., 1960).

The well documented extensive observations by Beasley and his colleagues (1959) and their comparative study of pleuropneumonialike organisms and ornithosis in pure and mixed infections (Beasley et al., 1961) deserve careful study by every poultry pathologist. Changes in the parenchymatous organs in the older birds were essentially proliferative, but they were necrotizing and proliferative in the younger turkeys. The most common cell type in the exudates was the mononuclear macrophage, invariably parasitized by psittacosis-ornithosis particles. In the spleen the reticuloendothelial cells, in the liver the Kupffer's cell, and in the seminiferous tubules the germinal epithelia are the host cells.

Of particular interest are the pulmonary lesions in experimentally infected turkeys because they are rarely a feature of avian ornithosis or psittacosis. Older turkeys dead from spontaneous ornithosis rarely have had pneumonic lesions, but young birds less than 2½ months old infected by the intratracheal route invariably had epithelioid pneumonitis, apparently originating at the primary bronchus extending to involve the portion of the lung ventilated by the bronchus. Just as in man (Lillie, 1933; Güthert, 1938), the monkey (Rivers and Berry, 1931), and the mouse (Kovac, 1961), the infiltration of normally inconspicuous stroma obliterates the respiratory tubules with varying amounts of fibrin, mononuclears, and septal cell proliferation and represents the reaction to the bedsonia in the lung of turkeys. The granulomatous nodules in the lumina of the tertiary bronchi in part explain why turkeys in the third to fourth week after infection are hypersensitive and exhibit cutaneous allergy to the ornithosis antigen. Infections caused by all members of the group induce allergy because they cause granuloma formation.

In contrast to the extensive focal inflammatory infiltrations in the myocardium seen in many fatal human infections (Scheidegger, 1961) it was affected in few turkeys despite extensive epicarditis with capillary hypertrophy. However, the wasted pectoral muscles of turkeys that have succumbed to experimental ornithosis may reveal myositis with extensive fragmentation of the fibers.

In the light of these gross and microscopic lesions, it seems important to search carefully for the cause of the "lower" form of turkey sinusitis, commonly referred to as air sac syndrome. Cultures alone should not be relied on. The similarity of the lesions of pleuropneumonia and pasteurellosis to those of ornithosis emphasizes the necessity for mouse or embryonated egg inoculations with tissues and exudates of diseased turkeys.

Seagulls (Larus ridibundus)

Little is known about the clinical picture or the lesions in gulls. The shot birds dissected by Meyer and Eddie (1952) showed no abnormalities other than the injuries due to penetration by the lead pellets. Strauss et al. (1957) illustrate and describe examinations of the lung of gulls from which they had isolated a bedsonia; they found a focal area of necrosis close to a small bronchus moderately infiltrated by monocytes. Reticulocyte reaction was noted in the spleen. Scattered necrosis and mononuclear granulomas admixed with polymorphonuclear leucocytes were in the liver, but since lesions were similar in a gull infected solely with salmonella they cannot be considered pathognomonic of ornithosis. According to Illner (1962b),

STEPS TO DETERMINE THE PRESENCE
OF PSITTACOSIS VIRAL AGENTS
INFECTED BIRDS (OR MAMMALS)

Pericardial exudate
Liver necrosis
Enlarged spleen
Exudate on intestines
Cloacal content

NORMAL

INFECTED

Smears stained for elementary bodies

Cloacal content

exudate spleen liver kidney

Emulsify and dilute with broth, with or without antibiotic solution in grinder—20 percent.

Culture on blood agar and enteric media

Refrigerator overnight

If heavily contaminated *centrifuge* or filter

Inject

Death → Sacrifice

5 mice

FIG. 23.8 — Graphic presentation of the technical procedures employed in the diagnosis of ornithosis or psittacosis in birds.

captured adult gulls (two of them) with no gross lesions yielded bedsoniae; *Pasteurella multocida* was cultured from tissues and exudates from those with symptoms of respiratory distress and fibrinous exudates on the pericardium and costal air sacs. Gull chicks may have enlarged spleens, and bedsonia with and without *Salmonella typhimurium* may be isolated from such spleens.

LABORATORY TESTS

Since ornithosis cannot be diagnosed clinically nor by gross anatomical inspection, the pathologist must use laboratory procedures (Meyer and Eddie, 1961) (Fig. 23.8).

Any material containing bedsonia must be regarded as highly infectious and dangerous to handle unless proper precautions are taken. Birds scatter infectious material in moist feces and nasal discharges attached to feathers and particles of down. Dead birds or birds killed with chloroform should be immersed completely in 5 per cent lysol solution; the bird should then be wrapped in lysol-soaked cheesecloth, frozen with dry ice, and preferably sent to a specially equipped laboratory. If the pathologist wishes to conduct his own examinations he should carry out the necropsies in a special room where there is no chance of infective material becoming dried. A special gown, rubber gloves,

and a suitable face mask with goggles should be worn by anyone in the examining room. Inoculated mice should be kept in glass jars with perforated metal lids. Diagnostic experiments on birds should not be undertaken by the inexperienced.

Smears prepared during necropsy from pericardium, serous surface of the liver, hepatic lesions, or spleen are fixed for 5 minutes with methyl alcohol and then stained with a reliable brand of Giemsa's stain for 5 to 20 hours (1 drop of stain to 5 mL of absolutely neutral distilled water). The stained impression on the slide may be differentiated rapidly in absolute alcohol, rinsed in water, dried, and examined (Fig. 23.9).

For rapid examination, the staining methods of Castaneda or of Macchiavello for rickettsia are very useful. Particularly the Macchiavello gives excellent preparations when used as follows: A 0.25 per cent solution of basic fuchsin in distilled water is prepared, and the pH adjusted to 7.1 with sodium carbonate. The tissue smear is dried gently with heat, and the fuchsin solution (dilute carbolfuchsin) is filtered over it through a coarse filter paper in a funnel. The fuchsin is left on the slide for 5 minutes and is washed off very rapidly by dipping the slide in a solution of 0.5 per cent citric acid held in a Coplin jar.

FIG. 23.9 — Intracellular colonies of ornithosis virus in pericardial exudate of a pigeon. Approximately ×1,200.
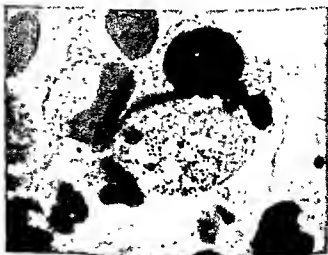
FIG. 23.10 — Section through chronic fibrinous plaque on pericardial surface of turkey recovering from ornithosis caused by bedsonia of low virulence. Slow, diluted and repeated Giemsa stain differentiated with acidified 70–80% alcohol. (Courtesy of Dr. R. A. Bankowski, Davis, California.)

The acid solution is washed off very rapidly with tap water. The smear is then stained for about 10 seconds with 1 per cent aqueous solution of methylene blue or 3 per cent aqueous malachite green (Mitscherlich, 1955). Excellent contrast stains can be prepared after a little practice; most intracellular and extracellular elementary bodies stain red, and cellular elements stain blue or green (Fig. 23.10).

Impression preparations of exudates stained with Giemsa solution are particularly instructive. Some investigators recommend the following staining (Stamp et al., 1950): Smears after fixing by heating are stained with dilute Ziehl-Neelsen carbolfuchsin (1:10) for 10 minutes and then differentiated very rapidly with dilute acetic acid lightly counterstained with dilute methylene blue. This modification is claimed to demonstrate the particles more distinctly than the Macchiavello method, and when smears stained by this method are examined by dark-field illumination, the bedsonia particles show as bright, pale green, round bodies.

Direct and indirect fluorescent antibody techniques have been used to see bedsoniae in peritoneal exudates of mice and impression preparations from air sacs of birds, but they were not successful in examining the enlarged spleens of chronically infected animals (Fig. 25.11). Impression preparations stained with an aqueous solution of acridine orange (1:50,000) and placed in a moist chamber show the particles in bright
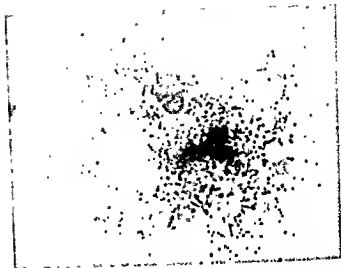
FIG. 23.11 — Elementary forms of psittacosis virus set free from a crushed cell. Peritoneal exudate of a mouse. Approximately ×1,400.

green against a background of dull luminescence (Neustroev et al., 1958, 1959). Results of examination of tissues from naturally infected birds have not been superior to those with the standard methods described.

In a comparative examination of spleen and liver from 44 ducks by the fluorescent antibody technique of Coons and the indirect complement fixation test, the former was more sensitive by about 30 per cent (Zelenkova and Strauss, 1963).

Similar procedures developed by Buckley and his colleagues (1955) for recognizing the intracellular psittacosis particles have been tried to rapidly diagnose ornithosis in turkeys. Explant monolayers of yolk sac cells were seeded with streptomycin-treated specimens of tracheal slime or cloacal content collected on swabs from turkeys suspected to be diseased. By applying the fluorescent antibody to such cultures, developmental stages of the bedsoniae could be recognized and identified early. A diagnosis of ornithosis could thus be rendered long before the mice or embryonated eggs yielded the agent (Donaldson et al., 1958). It is not known whether this method would be sufficiently sensitive and specific to be useful as a field test (Carski, 1961).

Material from birds with gross lesions in which elementary bodies can be seen with the microscope are excellently suited for isolation of the agent by inoculation of mice. Bacteria-free emulsions prepared from blood clots or fibrinous exudates are given to the mice by the intranasal, intracranial, or intraperitoneal route. Attempts to propagate the agent directly from infected birds in the yolk sac of the embryonated egg are not always successful; the parasite must first be enriched or enhanced in virulence by intraperitoneal mouse passage.

If the bedsonia cannot be seen through the microscope, for example in latent infections, separate pieces or pools of fragments of spleen, liver, and kidney of the suspected host are ground up with carborundum or sand to a paste and diluted with broth to a 20 per cent suspension. Cultures in blood broth deoxycholate medium and on blood plates must first be made in order to detect bacterial infections (PPLO, salmonellosis, streptococcosis, aspergillosis, and others). Then 0.5 ml. of the tissue suspension is injected intraperitoneally into each of at least 4 mice. Since isolates from pigeons, ducks, and other birds are only weakly pathogenic for mice when inoculated intraperitoneally, it is often necessary to establish infection by additional intranasal and intracranial inoculation of 0.03 ml. of the sterile suspension in the same animal at the same

time. Repeated intraperitoneal inoculation of the emulsion at 24-hour intervals may prove useful. It is preferable to treat contaminated suspensions with bacteriostatic agents; then pass them through collodion membranes with an average pore size of 450–600mμ (Meyer and Eddie, 1964).

Isolations of bedsonia from the peripheral blood and from fecal specimens are the most reliable and useful diagnostic tests.

Either defibrinated or freshly collected blood in heparin-rinsed syringes may be inoculated intraperitoneally in mice. The clot from a blood specimen carefully collected for serum yields the agent in many cases.

To isolate bedsoniae from excreta, droppings from individual birds or cloacal content from dead birds are suspended in broth or in 10 per cent horse serum in buffered water in the proportion of 1:3. Centrifuge at 500 times gravity for 10 minutes and treat with streptomycin sulfate in a concentration of 2 mg. per ml. of supernatant for several hours. The mixture is inoculated in volumes of 0.3 ml. intraperitoneally into groups of 3 to 5 mice, preferably 3 to 4 weeks old. The inoculated mice are then kept under observation; the spleens are removed from any that die and are emulsified and inoculated intranasally, intracranially, or intraperitoneally into a fresh pair of mice. Use of both the intracranial and intraperitoneal routes is particularly valuable. Those that survive are held under observation for 5 weeks; then they are sacrificed and their spleens used for further passage. Highly virulent material from turkeys causes death of the mice in 4 to 10 days; few mice recover. Material from pigeons, chickens, ducks, and some turkeys inoculated intraperitoneally proves fatal only to a few mice and long after inoculation. Repeated blind passages may be required to produce fatal infection or peritoneal exudates suitable for microscopic examination.

Large amounts of macerated suspected bird tissue emulsions may be inoculated intraperitoneally into mice repeatedly on consecutive days, and this enriches even specimens that cannot be freed of contaminants by antimicrobial drugs and may yield bedsoniae when use of the embryonated egg may fail.

The experimental disease in the mouse is not very characteristic; symptoms and signs consist of ruffled fur, apathy, closed eyes with discharge, and occasionally diarrhea. The incubation period and the severity of the disease depend on the amount of bedsonia in the test material, its infectiousness, its pathogenicity, the route of administration, and the strain of mice. If death occurs within 5 to 10 days the abdominal cavity is lined with exudate that covers the surface of the liver and is rich in elementary bodies. The liver may have undergone necrosis, and the spleen is enlarged. If death occurs between 15 and 30 days, the distended abdominal cavity contains a turbid effusion; the liver and spleen may be enlarged; elementary bodies are usually scant. Isolates of low virulence injected intranasally cause by the tenth day large foci of desquamative alveolitis accompanied by proliferation of interstitial elements (Kovac, 1961). Aggregates of lymphocytes around blood vessels, indicative of interstitial pneumonia, may lead to complete hepatization by the thirtieth day (Babudieri and Moscovici, 1955).

Bacteriologically sterile emulsions of the spleen and liver, in which the elementary bodies are rarely demonstrable, injected intracranially, produce choriomeningitis clinically recognizable by the paralysis they induce. Smears prepared from the meninges and choroid plexus usually show an enormous number of particles. They grow well in the yolk sac epithelium or when injected intranasally produce pneumonitis, the lesions of which furnish specimens suitable for microscopic examination. Demonstration of elementary bodies is considered adequate to establish a presumptive diagnosis of ornithosis. In pregnant mice bedsoniae produce extensive inflammatory and desquamative changes in the fetuses (Scheidegger, 1955).

Laboratories for the study of poultry

diseases may prefer the so-called chicken embryo technique. The tissues, properly macerated in broth and centrifugated, and the supernatant, treated with antimicrobial drugs, are used for inoculation of embryonated (6- to 10-day-old) eggs. The inoculum is introduced into the yolk sac or the allantoic cavity. Embryos dying after 48 hours are cultured for bacteria and the yolk sac membranes are examined microscopically. In the hands of persons skilled in handling embryonated eggs this test has resulted in primary isolations (Dane, 1955; Fagan, 1958). However, only experienced microscopists can identify the elementary bodies in properly stained yolk sac preparations. If tissues contain bedsoniae in low concentration and low virulence, they may fail to kill the embryos; repeated passage is then required.

For routine examinations the white mouse is the best indicator animal for the bedsoniae.

IDENTIFICATION OF THE BEDSONIAE

Field specimens or mouse material to be tested is held in lusteroid tubes at −70° C. until it can be examined. An agent with tinctorial characteristics and a development cycle typical of the group seen in smears prepared from the infected mouse, bird, or chicken embryo yolk sac, may be further identified through special tests.

Complement Fixation Test

Mouse spleen or yolk sac material rich in elementary bodies is converted into a coctoantigen according to the procedures described by Meyer and Eddie (1939a and b) or by Davis (1948) and then is used as an antigen in the direct complement fixation test with high-titered ornithosis antiserum obtained from naturally infected pigeons or produced in guinea pigs by inoculation with live bedsoniae.

Pathogenicity Tests

Inoculation of mice, guinea pigs, hamsters, parakeets, ricebirds, and pigeons with suitable dilutions of bedsonia suspensions by different routes is useful for distinguishing isolates from avian and mammalian sources; these tests separate, to some extent, the psittacine from the columban and from the turkey isolates (Meyer and Eddie, 1952). Most isolates from pigeons, ducks, and chickens have been of relatively low virulence for mice and have to be adapted by passage. As a rule, chicken, dove, and psittacine isolates induce fatal meningoencephalitis following intracerebral inoculation of pigeons. Duck isolates bring about temporary paralysis lasting for a week (Meyer and Eddie, 1952; Strauss, 1956).

Differences in virulence of bedsoniae, from different bird species for mice, the embryonated egg, and birds (particularly turkeys) are striking. Since these differences may reflect fundamentals of the ecology of ornithosis and evaluation of their infectivity for man, such systematic studies by the poultry pathologist are imperative (Page, 1959e).

In connection with pathogenicity and other tests, an estimate of the number of bedsonia particles, or elementary bodies, is essential. The counting method described by Smith and Manire (1959) comparing the number of particles with the number of polystyrene latex particles, under darkfield, phase contrast, or better, fluorescent light, is useful and reliable.

Cross-Immunity Tests

Subcutaneous inoculation of mice, once or several times, with sublethal doses of live or with large doses of killed bedsoniae and subsequent challenge with homologous or heterologous isolates may disclose resistance or susceptibility to certain isolates. While cross-immunity is definite, differences, corresponding roughly to those reflected in pathogenicity tests, become apparent. A weakly virulent isolate may immunize only against homologous challenge. Highly virulent isolates from psittacine birds or pigeons have given variable resistances against every isolate tested. Mice that survived immunization with the standard living virulent parakeet strain 6BC were resistant to 1,000 MLD of several pigeon isolates (Smadel et al., 1945a). The

results of these tests are difficult to interpret with the epidemiologic evidence or pathogenicity tests.

The Illinois pneumonitis strain, highly virulent for mice, has protected against two pigeon isolates (Shaughnessy, 1955). Guinea pigs that have recovered from infection with the egret or Borg strains have been resistant to intraperitoneal challenge with turkey isolates that cause death in control animals in 4 or 5 days (Meyer and Eddie, 1962a).

Lethality-Neutralization Tests

The results of this test have grouped 27 isolates into at least 6 separate groups (Manire and Meyer, 1950c). This technique is valuable in studies on the antigenic relationships of members of this group, but it is generally of little value in determining the exact source of an unknown bedsonia.

Neutralization of Bedsonia Infectivity

Antiserum prepared by hyperimmunization of roosters with known strains is mixed in the test tube with serial dilutions of the isolate to be studied. The mixtures are inoculated intranasally into mice. The animals are killed and examined on the tenth day, and the extent of the lung consolidation is scored according to the method of Horsfall (1939). Using this test, isolates may be classed into several distinct groups quite similar to those established with the toxin neutralization test (Hilleman, 1945).

When homologous toxic bedsonia-serum mixtures in proper proportions are injected intravenously, usually 50 per cent of the mice survive. The protection is quite specific for certain isolates, and several serotypes have been identified consistently. A hyperimmune serum prepared with a parakeet isolate renders 1×10^9 elementary bodies of the homologous strain noninfective. Carefully prepared serum cross-reacts little, if at all, with other avian isolates (Meyer, 1954).

In vitro neutralization tests of trachoma or inclusion blennorrhea in HeLa cell cul-

tures (Reeve and Graham, 1962) may prove applicable to ornithosis bedsoniae.

Serologic Differentiation

In preliminary studies a refined fluorescent antibody technique combined with cross absorption of serum has proved useful for distinguishing the trachoma agents. It may likewise be specific for ornithosis isolates (Nichols and McComb, 1962). Species-specific antigens in the cell wall of the bedsonia can be demonstrated in both the direct and indirect complement fixation tests (Ross and Jenkin, 1962). It is anticipated that once the techniques have been developed it will be possible to establish either the antigenic homogeneity or heterogeneity among isolates from certain avian species suffering from ornithosis. Many problems in the epidemiology and ecology of these infections could be elucidated if the specific antigenic structure of a bedsonia could be determined simply and accurately.

All bedsoniae share a heat-stable group antigen that fixes the guinea pig complement with the corresponding antibodies in the serum of man, monkeys, guinea pigs, cattle, sheep, psittacine birds, many pigeons, and ducks. They also produce heat-labile, type-specific antigens (for example, lymphogranuloma venereum and trachoma) in complement fixation tests with antiserum from which the group antibodies have been absorbed (Rice, 1961). The direct CF test for psittacosis was developed by Bedson and his colleagues (1935, 1937). It has been intensively applied to avian serum at the Hooper Foundation (Meyer and Eddie, 1939a, 1942; Meyer et al., 1939) and modified by others.

Antigens

Crude antigens have been prepared from the spleens or the pneumonic lesions of infected mice. More recently the peritoneal exudate has been used as a source material (Brand and Lippelt, 1954). Tissue cultures grown in Li and Rivers fluid medium or on Zinsser-Wei-Fitzpatrick solid medium (Meyer and Eddie, 1939a) or yolk

sac preparations (Smadel *et al.*, 1943b) are preferred by routine laboratories. Instead of infected yolk sac material, allantoic fluid rich in elementary bodies freed from anticomplementary substance by ether extraction (Davis, 1949), differential centrifugation, and lyophilization (Whitney and Gnesh, 1954) are preferred by some (Christensen, 1957). At the Hooper Foundation the following readily prepared antigen is used.

Inoculate 7-day-old embryonated eggs directly into the yolk sac with a suspension of a standardized virulent psittacosis strain. The suspension of the adapted psittacosis strain to be used is standardized so that most of the embryos die between hour 56 and 72 after inoculation with 0.25 ml. of a dilution of 10^{-4}. This requires a strain that is so adapted that a dilution of 10^{-8} to 10^{-10} kills the embryos. Harvest the yolk sacs as soon as possible after death of the embryos and examine them for elementary bodies; if rich in these bodies, pool and weigh. Grind thoroughly in a mortar with sterile sand and add beef heart broth pH 7.0 to make a 20 per cent suspension (a Waring blender presents risks). Culture for bacterial sterility. Hold in the refrigerator ($0° \pm 4°$ C.) for 3 to 6 weeks, preferably the latter. Centrifuge the emulsion for 30 minutes at 2,500 rpm to remove coarse particles, and put the supernatant in a heavy, sterile Pyrex flask and steam it in an Arnold sterilizer at 100° C. for 30 minutes or immerse it in boiling water for 30 minutes. When cool, add liquefied phenol to a concentration of 0.5 per cent. Permit the antigen to ripen in the refrigerator for at least a week. If the emulsion is kept sterile the antigenicity will be retained for at least a year.

A soluble phosphatide antigen (Volkert and Christensen, 1958) is potent, stable, and easily produced. The method of preparation (Volkert and Christensen, 1955; Jørgensen *et al.*, 1963) is as follows: fresh, heavily infected yolk sacs harvested on the third day after inoculation are boiled for 30 minutes over a water bath, and after cooling, are repeatedly extracted with 10 volumes of cool diethyl ether. The clear yellow ether extract is removed; the ether is distilled off on a water bath at 37° C.; a volume of warm acetone equal to the volume of ether is added to the oily residue; and the mixture is shaken and held at 55° C. for 1 hour. The acetone is decanted, and the precipitate freed from acetone by partial vacuum at 37° C. overnight. Buffered saline is used to suspend the fatty precipitate. This phosphatide antigen is extensively used in the Scandinavian countries (Jørgensen *et al.*, 1963) and the U.S.S.R. (Chervonskii and Popova, 1959).

A water-soluble group complement fixing antigen, efficiently extracted with sodium lauryl sulfate from purified suspensions of the meningopneumonitis bedsonia, has been found more active than the phosphatide antigen or the water-soluble coctoantigen (Benedict and O'Brien, 1956). The molecular configuration of this type of antigen renders the reactive site of the soluble antigen more readily available. Union of antibody and complement is believed to be firmer than with other bedsonia preparations. This type of antigen has been used with varying success in the direct CF test with turkey serum.

Interpretation of any CF or ICF reactions must be guided by the following:

In flock surveys when only a single serum specimen is tested, a titer of 1:4 or higher in the serum of pigeons, turkeys, chickens, ducks, or psittacine birds indicates either latency or recovery from infection. Carriers and active shedders may have high titers. Early in the infection, particularly in young birds, antibodies may not be demonstrable despite the presence of bedsoniae in the tissues. In a flock of pigeons a variable percentage of birds may harbor bedsoniae in spleen and kidney, but CF antibodies cannot be detected in the direct test. On the other hand, it may be impossible to isolate bedsoniae from birds with high titers. Flocks of pigeons yielding serum reacting in titers of 1:4 to 1:256 can be assumed to harbor infected birds. Invariably subinoculations of organs,

singly or in pools, have yielded bedsoniae. The antibody reaction pattern to bedsonia in pigeons has been described in detail (Monreal, 1958).

It is impossible to draw sound diagnostic conclusions on the basis of a test of one serum sample. If the test is being used to evaluate the results of chemotherapy, it is advisable to leg-band the pigeons and test the antibody levels monthly. The titer either remains stationary or rises to a peak and then gradually declines. The efficacy of attempted immunization may be followed by similar systematic examinations.

Persistence of antibodies in high titer for months or years is strong evidence that the bird is latently infected, even though attempts to isolate the bedsonia may be unsuccessful in the larger birds. A rapid or gradual drop in antibodies may indicate complete recovery and disappearance of the bedsonia from the viscera. Repeated serum tests, however, may fail to furnish titers reflecting the actual state of infection in an individual bird. Neither can the infectiousness of a pigeon or psittacine bird be determined from a series of serum tests.

Isolation of the bedsonia from the blood, droppings, or necropsy material remains the only conclusive method of diagnosis in individual birds, but this expensive, time-consuming, and destructive method need not be applied to detect infection in flocks. For this purpose the complement fixation test is reliable and inexpensive (Kissling et al., 1956).

The commercially available lymphogranuloma venereum antigen Lygranum is not a dependable test antigen in the diagnosis of ornithosis. Pigeon or human serum that reacts with a psittacosis antigen may fail to react with Lygranum (Eddie and Francis, 1942; D. J. Davis, 1955; Michael, 1957), thus the existence of a flock infection could escape detection if Lygranum were used. In tests of human serum referred to the Communicable Disease Control Section of the U.S. Public Health Service, 9.7 per cent of those positive in the CF test with a psittacosis antigen were

negative with Lygranum, and 61 per cent of the positive titers were lower than the corresponding titers with psittacosis antigen (Bucca, 1958). Other data are quoted that either antigen in 2 unit amounts is suitable for routine tests (Volkert and Christensen, 1954).

Two further limitations of this test in studying enzootics or epizootics of avian ornithosis or psittacosis deserve mention: (1) Handling and bleeding of the birds endangers those who are not immune. It should be entrusted only to persons with serologic evidence of a past infection or to those who take all of the necessary precautions against infection, including the wearing of properly constructed masks. (2) It may be difficult to secure enough blood to run the test from the peripheral vessels (wing vein or jugular vein) of small birds (Kissling et al., 1956).

The direct complement fixation test is useful in the rapid detection of flock infections in pigeon lofts and parakeet aviaries. The reactor rate in pigeons suspected as sources of human infections has differed significantly from that in groups of feral pigeons that have caused no apparent illness. The latter group has had fewer positive reactors and lower titers than the former (Dekking, 1949; Jansen, 1955). It is advisable to test pigeon serum simultaneously with both the direct and the indirect tests. In old pigeons the indirect test has yielded a higher percentage of reactors than the direct test. Isolation of bedsonia from the organs of reactors in the indirect test has been quite difficult. Apparently as the antigenic stimulus diminishes, inhibiting antibody is produced before all antibody demonstrable in either test finally disappears (Miles, 1954).

Modifications of the test for fowl serum have been recommended: Detergent-extracted antigens (Benedict and O'Brien, 1956) and the usual antigens fortified with normal chicken serum in the presence of turkey serum that have not been heated, but have been stored at $-29°C$, have yielded promising results (Brumfield and Pomeroy, 1957). Agreement between the

results of the direct and indirect tests using detergent-extracted antigen has been found (Neal and Davis, 1958; Page and Bankowski, 1960).

An extract antigen prepared from a Gram-negative coccoid bacterium closely related to *Bacterium anitratum* has been used in place of the specific psittacosis antigen (Volkert and Matthiesen, 1956). Because in the hands of several workers preparation of the *B. anitratum* has not been uniformly successful, the modification of the direct test is not recommended. Prolonged extraction of a saline suspension of a Herellealike bacterium has also been recommended as a substitute (Shimizu and Bankowski, 1963a and b). CF titers with the ornithosis or psittacosis antigen using Brumfield's direct CF test and ICF test were well correlated. Though not as sensitive as the ICF test for avian serum, this modification with a safe and stable antigen, if its reliability is confirmed, could be readily employed as an aid in the presumptive diagnosis of ornithosis in turkey flocks.

Indirect, or Inhibition of, Complement Fixation Test

Certain avian antiserums, including those from the chicken, duck, turkey, pheasant, and older pigeons, infected with bedsoniae do not fix guinea pig complement in the presence of homologous antigen (Rice, 1918, 1961; Karrer et al., 1950; Boulanger and Rice, 1954). Certain avain antibody-antigen complexes fail to activate the first component (C^1) of guinea pig complement; by substituting species specific avian C^1_1, the guinea pig C^1_2, C^1_3, and C^1_4 are fixed, and turkey, duck, and chicken serum may be examined in a modified direct CF test (Benson et al., 1961). The method has not been widely used because the ICF test has proved efficient, sensitive, and specific in diagnosis and serologic surveys (Karrer et al., 1950; Morimoto et al., 1958; Rindge et al., 1959; Page and Bankowski, 1960; Basova and Levi, 1960; Strauss et al., 1958; Strauss et al., 1960). The reaction in the ICF test is revealed by

adding specific CF indicator antibody (serum from a naturally infected pigeon or man or from immunized animals) to the chicken serum-antigen mixtures; if all the antigens have been used to saturate the inhibiting substance in the poultry serum, then none is left to combine with complement fixing antibody and complement yielding hemolysis. The rather difficult but valuable procedure for use in turkey and duck flocks is fully described (Meyer and Eddie, 1964), and an explanation of the reaction patterns has been advanced (Miles, 1954). The interpretation of the results is the same as that of the direct test. In routine tests with poorly collected serum from turkeys, the titer of 1:16 has been arbitrarily set as an indication of infection and should serve as a warning that the disease may appear clinically or anatomically on the processing line.

Microcomplement Fixation Test

Equipment and specific procedures for microtechniques have been refined and modified. Applied to the CF test and to the hemagglutination inhibition tests with standard reagents, the saving is eight-fold (Sever, 1962).

Hemagglutination Inhibition and Conglutinating Complement Absorption Tests

The hemagglutination inhibition procedure is simple, highly sensitive, and has the advantage of detecting complement fixing or complement fixation inhibiting antibody in bird serum (Hilleman, 1955); thus, both kinds of antibody may be detected in a single test. The hemagglutinin is apparently a phospholipid nucleoprotein complex, with lecithin being the agglutinating fraction and the nucleoprotein being the group-specific antigenically active component (Gogolak and Ross, 1955). One disadvantage is that the antigens have deteriorated during storage. The results have been less reliable as indicators of infection than the results of the ICF test (Bucca, 1958). The activity of the hemagglutinins has been well preserved when

the antigen is stored at $-20°$ C. or $-40°$ C. Some have found the hemagglutination inhibition test as useful a diagnostic test as the CF test (Matumoto et al., 1960). A passive hemagglutination reaction with tannic-acid-treated sheep red cells on which the meningopneumonitis protein antigen was adsorbed proved difficult to interpret, since correlation between the CF and hemagglutination reactions could not be discerned (Benedict and O'Brien, 1958).

The conglutinating complement absorption test is not satisfactory because the anticomplementary titer of the antigen preparation usually approaches the antigen titer. The results of both tests with pigeon serum have been somewhat erratic (Hilleman, 1955).

Agglutination Tests

This antibody reaction had been studied only to a limited extent (Bedson, 1932; Lazarus and Meyer, 1939; Labzoffsky, 1946), but interest in it has been renewed. Formalin-killed, purified and concentrated suspensions of elementary bodies prepared from infected mouse lungs (Babudieri and Secchi, 1952; Giroud and Jadin, 1954), or from allantoic or even yolk sac material (Neal and Davis, 1958) have been specifically agglutinated by serum from infected turkeys, chickens, parrots, parakeets, or mammals. Allantoic fluid, harvested 3 to 4 days after inoculation with a virulent turkey isolate, is formalized, centrifuged, and the elementary bodies are resuspended in $1/10$ volume in Sorenson phosphate-buffered water (pH 6) and dispersed by forcing the suspension through a 24-gauge needle by syringe. Giemsa's stain is added, and the suspension permitted to stand at room temperature for 36 to 48 hours. Excess stain is removed by several washings with distilled water at pH 5 to 5.5. The elementary bodies are finally suspended in phosphate buffer at pH 6.0 and dispersed again. Final concentrations are determined by titration against serum of known indirect fixation titer.

Agglutination has been detected by two methods—rapid plate test and the more re-

liable capillary tube technique (Mason, 1959). Serums from infected chickens and turkeys have given good coarse agglutination. The aggregates with pigeon serum have been finer, and with psittacine serum, somewhat intermediate. There has been satisfactory correlation between the results of ICF and agglutination tests, both slide and capillary tube, with turkey and chicken serum. The results seem to be specific for measuring antibody to ornithosis in turkey serum. Reactions at dilutions of $1:16$ or less are considered to be of questionable significance. Findings to date suggest that the capillary tube test may be a valuable aid in screening turkey flocks in epizootic areas before marketing or in conducting epizootiologic surveys. As a rapid test it is useful at autopsies when a few drops of serum separated from the clot in the ventricle of the heart may be mixed with the antigen and a reaction read within 30 minutes.

Antibodies demonstrable by the capillary tube agglutination and CF tests using detergent antigen appear early in the disease. Complement fixing antibodies persisted longer in the blood stream of infected birds than did agglutinins (Mason, 1959; Page and Bankowski, 1960). Testing of 93 serum samples collected during surveys of infected flocks indicated a close correlation (70 per cent) between the results of the CTA and ICF tests. The CF test with detergent antigen gave only a 40.2 per cent correlation; some turkey serum samples reacting in dilution of $1:256$ in the ICF test failed to fix the detergent antigen (Mason, 1959; Meyer and Eddie, 1960, unpublished). For a practical application it must be emphasized that the serologic reactions determined by any one of these three reliable procedures (CTA, CF, ICF) usually do not become detectable for at least 10 to 16 days after infection. Early detection of ornithosis in a flock is of greatest importance in order to control spread of the disease and to prevent human infection. A sensitive specific test, for example, the capillary agglutination procedure or even a slide agglutination test,

can prove the existence of active infection with gross anatomical lesions several days before the bedsonia is isolated. Lack of a practical standard antigen has prevented routine laboratory use.

Intradermal Tests

Crude detergent extracts of the meningopneumonitis agent have been studied experimentally in chickens for the purpose of developing an intradermal test to detect ornithosis (Benedict, 1958a). Birds experimentally infected for less than 3 weeks showed either no reactions or reactions of varied intensity (Benedict et al., 1955; Benedict and McFarland, 1956b). The skin test used in naturally infected flocks yielded 85 per cent and the ICF test 92 per cent reactors, or 85 per cent agreement; in another less severely diseased group the agreement was 76 per cent (Benedict and McFarland, 1958). The claim by Soviet investigators and others that intradermal testing is the most practical for extensive ornithosis surveys is open to question (Terskikh, 1957a; Terskikh et al., 1962). For epizootiologic surveys the skin test requires repeated handling and examination of birds, some of which may be infectious and pollute the environment in which the personnel is exposed during the testing. The skin test to diagnose the infection in individual birds or man is unreliable; indeed it would be subject to the same criticisms as have been made against the intradermal test for diagnosis of other infections in man and animals. Individual birds and man may react though they were never infected with bedsoniae.

IMMUNITY AND ACTIVE IMMUNIZATION

The immunity is not completely understood. The immunity of many parrots after an attack is nonsterile, and it was formerly believed that persistence of the psittacosis agent was obligatory for the immunity. More recent studies indicate that latency is the sequel to incomplete or delayed autosterilization, due to host cell adaptation to new variants of the bedsoniae (Officer and Brown, 1960). In fact, considerable evidence supports the idea that the resistance is an innate constitutional one, reinforced by a short-lived infection. Individual squabs, chicks, parrots, or parakeets may resist massive injection of bedsoniae, readily destroy them and never become carriers. Others either succumb to small doses or recover and the infective agent remains in their organs for years. Since constitutional factors determine the ability of birds to resist infection, it should be possible through selective breeding to develop strains of birds that will not acquire a nonsterile immunity, so eminent a source of persistent infection in lofts and aviaries (Meyer and Eddie, 1962a).

Formalin-treated or ether-extracted, inactivated, concentrated bedsonia suspensions have conferred considerable immunity to mice, but have not completely prevented multiplication of the infector (Bedson, 1938; Meyer et al., 1942b). Mice hyperimmunized by the intraperitoneal route with killed 6BC and Borg bedsonia have resisted 10 homologous or heterologous intracerebral or intranasal LD_{50} (Wagner et al., 1946). Effective immunity has been established when immunizing and challenging doses have been given by the intraperitoneal route (Yanamura and Meyer, 1942). Concentrated tissue cultures treated with formalin, administered intramuscularly in as many as 7 injections, have protected ricebirds and parakeets against homologous challenge of 100 MLD. The immunity has not been strong enough to protect against massive challenge. Preliminary experiments failed to encourage hope that a practical safe method of active immunization of parakeets with inactivated bedsonia could be developed (Meyer et al., 1942b).

Hughes (1947) vaccinated alternate pigeons from the same nest when they were 3 weeks old and again 3 weeks later with a formalized, inactivated yolk sac pigeon ornithosis antigen prepared according to a modification of a method worked out by Smadel et al. (1913b). When the birds vaccinated by Hughes were exposed to natural infection for 4 months, 6 vaccinated

birds and 5 in the control group died of ornithosis.

In ewes, formalin-inactivated enzootic abortion bedsonia eliminated abortion if the vaccine, prepared from infected ovine fetal membrane, was incorporated in an adjuvant (McEwen *et al.*, 1955; McEwen and Foggie, 1956). This suggests that further efforts with vaccines in adjuvant might be rewarding.

In a critical review on the immunity against bedsonia infections several pilot experiments are described that suggest that psittacine birds could be immunized with concentrated formalin-inactivated psittacosis vaccine in adjuvant (Meyer and Eddie, 1962a). In search of a way to prevent acute ornithosis in turkeys, and in doing so reduce the risk of occupational infections, vaccination with formalin-inactivated vaccines in adjuvants was evaluated with the following results: (1) Three intramuscular inoculations 1 month apart conferred some immunity against intratracheal infection with a highly virulent strain: there were still gross lesions in a third of the turkeys sacrificed on the thirtieth day after challenge. The bedsonia was recovered from 14 of 101 vaccinated turkeys. Bedsonemia on the tenth day after challenge was, as a rule, not demonstrable in vaccinated birds. (2) Half of the unvaccinated had extensive gross lesions and the inoculated agent was recovered from 20 (47 per cent) of 42 surviving unvaccinated turkeys. (3) A single inoculation gave less protection. Concentration of the bedsonia doubtless would enhance immunogenicity, but with present production methods it would increase the cost beyond practicability (Meyer and Eddie, 1962a).

In the few studies in which it was tried a more effective immunity has been achieved with living bedsonia vaccines. In guinea pigs inoculated intradermally with virulent psittacosis or murine pneumonitis isolates, resistance to intratracheal challenge was developed against 40 respiratory LD$_{50}$ (Wagner and Victor, 1953). In earlier experiments (Rivers and Schwentker, 1934) monkeys were immunized against intra-

tracheal inoculation by intramuscular injection of small doses of a virulent psittacosis strain. Active immunization of fowl with living attenuated bedsonia even if successful is not practical and is of doubtful value in the control of ornithosis.

CHEMOTHERAPY

Early studies of the effects of antimicrobial drugs on certain bedsoniae revealed that they delay or stop multiplication but are not completely bedsonicidal. Tetracycline compounds have been effective in treatment, given in the dosage of 0.8 mg. in 2 injections daily for 14 days (Meyer and Eddie, 1954b and 1955). In chick embryos chlortetracycline is five times as effective as chloramphenicol on a molecular basis, and three times as effective on a weight basis (Cox, 1955). Chlortetracycline is the preferred drug for human infections caused by ornithine and psittacine bedsoniae (Fitz *et al.*, 1955). Symptomatic improvement in man is commonly prompt if treatment is begun immediately after the onset with 1 to 2 gm. daily and is continued long enough. Inadequate treatment or exceptional susceptibility may be followed by relapse. In one exceptional outbreak, broad-spectrum antimicrobial drugs did not control psittacosis contracted by infants through contact with pigeons (Berman *et al.*, 1955). It may be that in these cases the drugs, being only bedsonistatic, received little or no support from immunogenesis, which failed in the malnourished infants suffering from gastroenteritis and dehydration.

Early studies on treatment of latent avian psittacosis and pigeon ornithosis were discouraging, but in a later one daily parenteral or oral therapy with up to 100 mg. of tetracycline compounds per kilogram of body weight has improved the health of individual pigeons, reduced mortality, curbed epizootics, and reduced the hazard of infection in aviary personnel (Meyer and Eddie, 1955; Arnstein *et al.*, 1964). It does not always eliminate the carrier state in every bird, but if treatment of backyard flocks is repeated at intervals of one month,

the infection may be ultimately eradicated (Meyer et al., 1958).

Several field trials to eradicate ornithosis from squab farms with tetracycline compounds were disappointing. Intensive treatment by injection of aqueous solution or sesame oil preparations with tetracycline failed to free the latent carriers, or the isolation procedures were inadequate and favored heavy reinfection (Meyer and Eddie, 1955). Under controlled laboratory conditions the mortality rate fell during administration of chlortetracycline in a concentration of 80 gm. per gallon of water for a month, and squabs did not become infected. However on commercial breeding farms where chances of reinfection from a heavily soiled environment are ever present, it suppressed the bedsonia activity only temporarily and some birds have remained carriers (Shipkowitz et al., 1958).

Pigeons will eat a chicken scratch feed containing crude chlortetracycline SF 66 (4 per cent by weight of the feed). In small pilot experiments on naturally infected pigeons, 700 mg. of chloramphenicol per bird, administered orally continuously or interruptedly for 7 days, exerted a promising therapeutic effect (Jansen, 1955; Fritzsche et al., 1956). Parenteral administration of this drug is claimed to cause shock and death in pigeons; such side effects have not been observed when tetracycline compounds were used.

In a field trial clinically healthy but latently infected (serologically proved) 5- to 6-week-old ducklings (85 per cent infected) were offered feed that contained 0.5 mg. of chlortetracycline/gm for 11 days Only traces of the drug could be found in the blood during treatment. When the treated ducklings were dressed, 1 of 11 women employees (15 to 53 years old) contracted a subclinical infection (16-fold rise in titer). The 4 pools of spleen and liver prepared from 48 treated birds yielded the ornithosis agent. The 500 untreated control ducklings caused no infections, and only 2 of 56 organs pooled were proven infected with the agent (Strauss et al., 1961).

Extensive experiences in the treatment of psittacosis in parakeets and parrots suggest that the dosage and duration of chemotherapy were inadequate, particularly since the holding of the birds in large groups offered opportunity for continuous reinfection.

Experiences with chemotherapy of parakeets encouraged the use of tetracycline compounds to suppress ornithosis in turkeys. In 1952, epizootic ornithosis was treated with chlortetracycline at concentrations of 100 to 400 gm. per ton of feed. The epizootic subsided, but processing personnel contracted the infection in several instances. Subsequently pilot experiments with artificially implanted turkey poults were reported. Treatment with 400 and 800 gm. of drug per ton was instituted immediately for 7 days after infection and continued for 14 days, and this completely suppressed the infection (Davis and Delaplane, 1955). In a natural outbreak turkeys were given supervised treatment with 200 gm. of chlortetracycline per ton of feed for 3 weeks. Infection was suppressed, but at the time of processing, 288 of 1,856 toms were condemned because of aspergillosis. Residual lesions of ornithosis were present, but bedsonia was not recovered by the chicken embryo technique from tissues removed at the time of slaughter (Davis and Delaplane, 1958b). Treatment of epizootics in Minnesota caused by bedsonia of low virulence was also successful (Pomeroy et al., 1957).

These successes could not be repeated in the Oregon outbreaks. Three flocks were treated, during a progressive epizootic, with chlortetracycline and oxytetracycline at a dose of 200 to 400 gm. per ton of feed. The clinical response was favorable, but bedsoniae were isolated from organs of treated turkeys when processed after 32 or 39 days of continuous treatment. All 3 treated flocks, when processed, caused human infections. In systematic studies on normal and infected turkeys under supervision at a poultry experiment station the oral ad lib. intake of pellets containing 221 to 354 gm. of oxy-

tetracycline per ton failed in some cases to yield demonstrable antibiotic levels in the blood or organs, but these were found in the cloacal contents. The fecal material contained 96 to 480 micrograms of drug per gram. Therapeutically effective levels have been found in turkeys when the mash contained 2,800 gm. per ton and the calcium level of the feed was reduced with a chelating agent (6 gm. of sequestrin/pound of feed).

Because present knowledge is incomplete, no recommendations can be made. It is not unlikely that tetracycline compounds in economically practical dosage may be used to prevent severe infections and epizootics.

In transmission experiments ornithosis did not spread from artificially infected to healthy poults when the infected birds were fed 100 gm. of chlortetracycline/ton of feed. Beginning on the second, third or fourth day after intratracheal inoculation of 0.5 ml. of chick embryo material infected with a virulent Texas isolate, poults were offered feed containing 200 gm. of drug per ton. The infection was apparently aborted, mortality prevented, gross lesions were suppressed, antibodies did not develop, and the infected turkeys proved susceptible to reinfection. In another study adult turkeys that recovered from ornithosis with or without tetracycline therapy developed significant levels of ICF antibodies and proved resistant to subsequent challenge (Davis and Watkins, 1959). Oxytetracycline (terramycin) was unsatisfactory for therapy at 200 gm/ton, whereas this dose of chlortetracycline did eliminate the agent from the tissues. When the birds were fed 600 to 800 gm/ton for 3 weeks, infection was completely prevented. Terecephalic acid and low calcium diet enhanced the chemotherapeutic efficacy of tetracycline (Moore and Watkins, 1960). A crucial experiment to test the prophylactic efficacy of chlortetracycline in the low level of 10 gm/ton, an amount commonly incorporated in turkey feed for growth promotion under field conditions, has not been made. This low level has no inhibitive effect on the course of the infection induced by intratracheal infection even when treatment was begun immediately after infection (Davis and Delaplane, 1955).

Another later report (Francis, 1960) of a field observation is encouraging: A flock of 2,200 Broad-Breasted Bronze turkeys, 6 to 9 weeks old, lost 130 to 150 birds during the week before ornithosis was diagnosed. On the fifth day after treatment, with feed containing 200 gm. of chlortetracycline/ton being offered, the daily mortality dropped to 1 or 2 turkeys a day, and after 3 weeks no further deaths occurred. The epizootic was definitely arrested, and the total mortality in the flock was maintained at 300 birds. Of interest is the observation that drug intake for at least 3 weeks was required to arrest the ravages of the ornithosis agent.

Prolonged feeding of 200 to 400 gm. of drug per ton of feed has not freed the tissues of all diseased turkeys from bedsoniae. The infective agent has continued to be a risk to the processor, and birds not thoroughly and carefully eviscerated might endanger the person who prepares them for cooking. Some bedsoniae have acquired drug resistance in a variety of hosts; their tendency to chronicity increases their potentiality for developing drug resistance. This possibility is especially significant in connection with programs designed to eradicate ornithosis from poultry or psittacosis from psittacine birds by chemotherapy (Gordon et al., 1957). Apparently this risk is not great; several isolates from the organs of turkeys treated for 3 months and of parrots for 50 days have been drug sensitive (Meyer and Eddie, 1958b; Arnstein et al., 1963, unpublished).

EPIDEMIOLOGY

An estimate of the number of human infections acquired from contact with poultry is given in Table 23.1. In the United States between 1952 and 1956 the source of diagnosed psittacosis was sought in only half of the cases. In 1955 neither the occupation of the patient nor the source of infection was determined in 191

(57 per cent) of 333 cases (Andrews, 1957). Mild infections characterized by only malaise and minor respiratory symptoms undoubtedly go undiagnosed.

The sources of infection in man have changed since the outbreaks of 1929 and 1930 (Roger and Lépine, 1961).

Birds of Pleasure and Show

Since 1876 these birds have caused 4,000 reported apparent or inapparent infections. Exposure to cage birds in the home or occupational contact by bird breeders, dealers, pet shop owners, and employees in zoological gardens have been documented.

Oceanic Birds

Petrels were responsible for human outbreaks, principally among adult women on the Faeroe Islands; occasional infections have been traced to sea gulls (*Larus* sp.) and muttonbirds (*Puffinus tenuirostris*); egrets are suspected in the Louisiana outbreak of psittacosis. Human disease on the Faeroe Islands stopped when the killing and preservation of fulmars for food was discontinued.

Pigeons

Human psittacosis of pigeon origin has been traced to pigeon lofts or to flocks of racing pigeons (Meyer and Eddie, 1942; D. J. Davis, 1955). On the basis of serologic studies (Mohr, 1954; Meyer, 1958) it is estimated that around 50 per cent of pigeon breeders have significant serum titers. Few cases attributable to contact with feral pigeons have been reported. Pigeons in parks and streets constitute some hazard to people who come into contact with the dust from dried droppings (Levinson *et al.*, 1944; Mach *et al.*, 1950; Fallet, 1951; Lépine and Sautter, 1951; Fischer, 1955; D. J. Davis, 1955; Shaughnessy, 1955; Weyer and Lippelt, 1956; Babudieri, 1956; Berman *et al.*, 1955; Ephrati-Elizur and Bernkopf, 1956; Parry and Griffith, 1962). The history of exposure often incriminates dust of pigeon-roosting or nesting areas without direct contact with the birds

(Cohen *et al.*, 1946; Boucher and Sautter, 1951). The cleaning of wooden houses holding homing pigeons in the back yard of urban or rural dwellings constitutes an important risk (Meyer and Eddie, 1942; Dekking, 1950; Dekking and Ruys, 1951; Ellenbogen and Miller, 1952; Bacon, 1953; Deyke and Meyer, 1955; Schoop and Kauker, 1955; Semple, 1956; Monreal, 1959; Jansson, 1960).

Although there seems to be no evidence of widespread epidemic human infection of pigeon origin, the reports of fatal infections in infants (Berman *et al.*, 1955) and the relatively high incidence of sporadic, subclinical, mild-to-severe infections among pigeon fanciers and dealers indicate a public health hazard (Dekking, 1950; D. J. Davis, 1955; Shaughnessy, 1955; Parry and Griffith, 1962). In flocks responsible for human infections there has been a high proportion of reactors in the complement fixation test, and the ornithosis agent has been isolated from 50 of 60 and from 16 of 18 pigeons examined (Mohr, 1954; Kemmerer *et al.*, 1956). For enzyme studies two biochemists and two animal caretakers using the organs of one of seven pigeons all proved infected by isolation of the bedsonia, and students making psychological observations on visibly sick birds acquired confirmed clinical and subclinical infections. These group illnesses attest to the high contagiousness of pigeons in the acute stage of ornithosis. Repeated surveys of pigeons held for experiments in laboratories revealed CF titers greater than 1:32 in 30 to 50 per cent of the tested birds (Meyer and Eddie, 1947–62, unpublished observations).

Chickens

Infection has been contracted from chickens only when contact has been very close (Meyer and Eddie, 1942; Karrer *et al.*, 1950a; Duncan *et al.*, 1952). The recognition of a single case of psittacosis in a poultry processing plant in Connecticut led to an industrial survey and the discovery of 12 of 50 employees with high serum titers particularly among those hav-

ing direct contact (eviscerations) with chickens (Rindge et al., 1959).

Ducks and Geese

Histories of mild cases of atypical pneumonia among duck handlers were identified as psittacosis on Long Island (Korns, 1955) and in Virginia (Andrews, 1957). The many recent reports on human ornithosis in Central Europe document that ducks and geese play the same public health role in Czechoslovakia (Strauss, 1957; Strauss et al., 1961; Serý et al., 1961), East Germany (Kukowak, 1961a and b; Kukowak et al., 1960; Siegmund, 1960; Kielstein, 1961; Ortel, 1961, 1963), Austria (Fürst et al., 1957), Hungary (Derzsy, 1958; Varnai et al., 1960; Dömök, 1963), Poland (Parnas et al., 1961), Bulgaria Kuyumgieff, 1957), Roumania (Sarateanu et al., 1960), and the U.S.S.R. (Terskikh, 1954, 1957b) as turkeys have in the United States. In rural areas during small outbreaks, workers on small poultry breeding and fattening farms and then employees in poultry-processing plants are victims of the infection. Dr. Vladimir Serý contributed Tables 23.6 and 23.7 showing the places and numbers of human infections and the avian sources suspected of causing 1,072 cases of psittacosis

in Czechoslovakia during the past 10 years. Intimate contact with diseased ducks during picking and processing caused at least four times as many occupational illnesses in poultry plants as on farms. This relationship, conditioned by the more intimate and continuous exposure to the duck ornithosis agent, is illustrated by slightly different findings in a study of ornithosis in eastern Slovakia (Strauss et al., 1960). On a duck farm on which 1,350 of 7,000 ducklings died, 8 of 10 employees contracted psittacosis; 6 patients had pneumonitis and 2 had influenzalike attacks. Serologic surveys among 213 persons on 111 small poultry farms and poultry hatcheries revealed only 24 significant antibody titers. Twenty-one of the reactors who had histories of clinical illness (11 hospitalized: 8 pneumonitis, 2 influenza, 1 typhoidlike illness) belonged to the feeding and tending personnel in close contact with the ducks. Just as observed during the epidemiologic studies in Bohemia (Serý et al., 1957), only 3 members of duck-keeping families, without histories of illness, reacted with significant CF titers.

The Czechoslovakian epidemiologists stressed that ornithosis was relatively frequent in young women, and that it is

TABLE 23.6

PLACES OF INFECTION AND PROFESSION OF HUMAN CASES OF ORNITHOSIS IN CZECHOSLOVAKIA (1953-1963)

Place of Infection	Professions of Human Cases	Number	Total
Poultry Industry	Employees	835	847
	Accidental visitors	8	
	Veterinarians	4	
Small poultry farms of individual breeders	Keepers and members of their families	179	181
	Accidental visitors	2	
Poultry shops	Merchants	4	4
Kitchen	Cooks	2	2
		6	6
Laboratories	Laboratory workers	32	32
Not indicated	Different professions		1,072
Total			

Two cases were suspected to be caused by interhuman transmission: a dentist's aide and a roentgenologist who became ill while diagnosing several cases of ornithosis during the epidemic period.

TABLE 23.7

POSSIBLE SOURCE OF HUMAN CASES OF ORNITHOSIS IN CZECHOSLOVAKIA (1953-1963)

Possible Source of Infection	Number of Cases		Number of Human Cases
	On poultry farms	Outside poultry farms	
Ducks	313	165	986
Ducks and other poultry	489	19	
Geese	30	1	523
Geese and other poultry	475	17	
Chickens	14	2	200
Chickens and other poultry	172	12	
Turkeys	1	0	72
Turkeys and other poultry	66	5	
Pigeons	0	5	5
Pheasant (imported)	0	1	1
Not indicated	0	32	32

not always benign. The two-phase course of the disease is frequently complicated by meningeal manifestations and myocarditis.

More important are the frequent recrudescences that, according to Serý (1962), were recorded in 94 patients within 6 months, but more often within 1 month among 1,072 ornithosis cases. Many were relapses, since once the patients had had the disease they avoided contact with infected poultry. On the other hand, in 19 persons the attack 6 to 60 months after their first attack must be considered reinfection. They occurred during the annual seasonal outbreaks and only in workers who returned to the heavily contaminated environment of the farm or processing plants. Recrudescences were reported in persons who discontinued work in the poultry industry. During an outbreak of ornithosis in East Germany due to contact with ducks, 56 of 70 treated patients had one to three or more relapses from the first to third week after the body temperature had returned to normal (Gneuss and Koitzsch, 1961). Chloramphenicol and tetracycline in the total dose of 15 gm. (daily intake of 1.5 to 2 gm. for 7 to 10 days) was probably inadequate. Other factors, perhaps an unusual bedsonia, may

have been responsible for the high incidence of the relapses. Reinfections may reflect early treatment with antibiotics, hence no immunity conferred by the infections; they are not confined to the duck ornithosis agent but occurred in persons infected with psittacine and turkey agents (Meyer and Eddie, 1956c and 1962a; Irons et al., 1955). Care of recently hatched ducklings and cleaning of pens on a duck farm caused 6 cases of psittacosis in Austria (Fürst et al., 1957). The sporadic cases during April through June, 1945, on Long Island were all in persons who came in intimate contact with ducks (Korns, 1955).

Turkeys

The epidemiology of the explosive outbreaks due to exposure to sick and grossly diseased turkeys deserves detailed discussion. Since 1948, and particularly since 1952, persons processing adult turkeys have contracted ornithosis (Table 23.8). The CF titer in the tested sera of the workers who were ill increased 4- to 8-fold. Five outbreaks, consisting of at least 96 cases with 7 deaths, occurred in Giddings and El Campo, Texas, in 1948, 1951, 1952, and 1953. All illnesses affected workers who had been dressing young turkeys for the Thanksgiving or Christmas market or had

TABLE 23.8

PSITTACOSIS IN MAN CONTRACTED THROUGH CONTACT WITH INFECTED TURKEYS
NOVEMBER, 1948–DECEMBER, 1961
(Hooper Foundation Records)

Date	Place	Cases Officially Reported	"Additional Suspected Cases"	Deaths
	Texas			
November, 1948	Giddings	22	0	3
December, 1951–January, 1952	Giddings	48	0	4
April–May, 1952	Giddings	19	0	0
November–December, 1952	El Campo	8	0	0
	Corsicana	48	0	0
April, 1954	Corsicana	40	2	0
April, May, June, 1954	Lampasas	71	9	0
	Brady	24	6	0
April, May, June, 1954	Taylor	6	5	1
May, 1954	Comanche	0	5	1
May, 1954	Austin	26	8	0
September, 1956	Houston, Marlin	13	0	1
December, 1957–January, 1958	Lampasas			
	New Jersey			
December, 1954	Dutch Neck	17	0	1 (?)
	Oregon			2
January–May, 1956	Portland, Salem	86	0	0
December, 1957	Portland	1	5	0
February–December, 1958	Portland, Salem	1	6	0
January–December, 1959	Portland, Salem	3	11	0
January–December, 1960	Portland, Salem			
January, 1957	Pennsylvania	9	1	0
August–December, 1956	Wisconsin	7	10	0
January, 1958	Wisconsin	4	0	0
1956	Washington			
May–June, 1957	British Columbia	27	0	0
	Vancouver	1	0	0
January–December, 1960	California			
	Oregon	3	0	0
	Portland, Salem	1	0	0
	South Dakota			
	Texas			
January–December, 1961	Dewitt, Yoakum	17	5	0
December, 1961		510	68	11 (12)

been handling adult birds marketed at the end of the egg-laying season. Illnesses ranged in severity from minor influenza-like attacks to fatal toxemia. In most cases in which X-rays were made, evidence of pneumonitis was observed.

The clinical attack rate in 135 presumably exposed workers was 33.6 per cent, despite the fact that scarcely more than three years earlier an outbreak of psittacosis had occurred in workers in this plant. Eight of 22 persons who had psittacosis in 1948 were again exposed as pickers or eviscerators in 1951. Five escaped (apparently immune) but 3 suffered reinfection.

Nine others apparently exposed in 1948 became infected again in 1951 or 1952.

Since 1952, use of broad-spectrum antimicrobial drugs has seemed beneficial, but relapses have been frequent in treated patients. In an outbreak from April through July, 1954, over 264 persons were infected, and one of them died (Irons et al., 1955). No cases were reported from September through December that year, and in the period there was an apparent low incidence of visibly diseased turkeys on the processing lines. Protective measures adopted by some plants may have helped keep the incidence low. The extent to

which acquired immunity of the workers might participate would be difficult to learn In 1965 the National Office of Vital Statistics, Public Health Service, U.S. Department of Health, Education, and Welfare, listed only 1, possibly 2, human infections resulting from contact with turkeys in Texas.

The processing of visibly diseased turkeys in California in 1954 did not cause infection in workers in the plant. Of 88 employees tested, the sera of 3 gave CF reactions. Sera from residents or employees of the turkey ranch where the infected flock was raised did not give CF reactions This is the first instance in which a bedsonia isolate of low virulence was found.

A thorough study of several outbreaks in Oregon disclosed the following: Of 13 workers on a farm where many birds had died of ornithosis, 2 were ill and I had died in February 1956. Of 9 workers on the other affected farm, 3 were ill when the epidemiologic investigations were undertaken The serologic reaction of 1 was positive and of the other 2 suggestive of psittacosis. At a rendering plant where dead turkeys from both farms were taken, 70 per cent of the employees (5 cases confirmed, 21 probable) had ornithosis. In a private home where some of the turkeys were dressed, the husband contracted severe psittacosis pneumonitis, proved by isolation of the bedsonia, and died; the wife recovered from a respiratory infection and had a CF titer.

During May, 1956, of 102 workers in a processing plant where apparently healthy turkeys from a third nearby farm were procesed, 52 developed signs of ornithosis. Complement fixation tests were positive in 7.

Of 86 persons who became ill (2 deaths) in the 1956 outbreaks in Oregon, 5 worked on turkey farms, 55 were employed as poultry processors, and 29 were employed in rendering plants. Hospitalization was required by 28, sometimes for several weeks. The infection was believed to have been air-borne, because many of the affected persons did not have physical con-

tact with turkey carcasses (Scruggs, 1957) The rendering (converting diseased carcasses into fertilizer) did create infective aerosols, as subsequent studies demonstrated (Spendlove, 1957). Diagnosis of psittacosis was based on the results of serologic tests interpreted with clinical and epidemiologic data. A total of 313 persons in contact with diseased turkeys or turkey products or exposed to aerosols and dust created by the handling, processing, or disposing of such birds, who contracted an illness like psittacosis were classified as confirmed, probable, or possible cases. Persons in direct contact who had no illness, but in whom a 4-fold or greater rise in CF antibody titers was demonstrated were classified as inapparent infections. Only 9 of the 95 serologically recognized infections were subclinical or inapparent. The attack rate ranged from 21.8 to 48.8 per cent in processing plants and was as high as 71 per cent in rendering plants. Exposure to a highly virulent bedsonia induced a higher incidence of clinical than subclinical infections.

In this respect it differed from that noted in poultry plants in which only ducks, geese, and chickens are processed. Though psittacosis acquired through exposure to ducks may be just as severe as psittacosis contacted from psittacine birds or turkeys, the relationship of 1 clinical to 2 subclinical infections lends some support to the contention that infections originating from water fowl and other poultry are milder. Generalization should be avoided, but the several observations (Graber, 1957, 1959; Meyer and Eddie, 1962a) imply that clinical cases are fewer when workers are exposed to turkeys infected with ornithosis agents of low virulence than when the strains are highly virulent.

Epidemiologists trying to analyze occupational ornithosis in processing plants have found it difficult to interpret the antibody titers of the employees since it was soon recognized that a variable percentage had titers at the time they contracted the illnesses that clinically were not typical for psittacosis. During the outbreaks

in Oregon, blood specimens routinely taken from the employees in several processing plants before they became involved in outbreaks furnished a serologic baseline that aided in diagnosing the illnesses among the workers.

Observations on serologic baselines led to the suggestion that a rough estimate of the degree of exposure or the incidence of ornithosis in poultry flocks at certain times of the year could be obtained if serologic tests were made at frequent intervals (McCulloh, 1955; Rosen, 1955; Osgood et al., 1956; Dickinson et al., 1957; Graber, 1957; Hines et al., 1957; Rindge et al., 1959). This reasoning is justified because with few exceptions illness among the employees has served as a sentinel for the unrecognized or often incorrectly diagnosed epizootics in turkeys that came to slaughter. Baseline antibody reactions are equally important to support decisions about whether an illness in a plant worker is acute psittacosis, a reinfection, or a relapse and whether he is entitled to compensation.

MODE OF TRANSMISSION

There are three known pathways of transmission from bird to man which are in order of importance, (1) through circulation of the bedsoniae in the air (Fig. 23.12), (2) through handling sick or dead birds or having contact with feathers, excreta, or nasal discharges of sick or latently infected birds and (3) through bite wounds. The ease with which inhalation of bedsoniae induces pneumonic lesions in susceptible mammals points directly to the respiratory tract as the principal entry. Handling of sick or dead birds or contact with feathers soiled with infective discharges or excreta or with droppings from latently infected flocks—all capable of creating bedsonia-carrying aerosols—are fully proved modes of infection (Rugiero et al., 1950; Mumme, 1955; Osgood et al., 1956; Spendlove, 1957). In experimental studies on primates (McGavran et al., 1962) the psittacosis agent was seen to be impinged along the respiratory bronchiole. It spread, perhaps along the lymphatics,

and lobular and confluent lobular pneumonia resulted from extension of the single focus. Recovery of bedsoniae from the blood, liver, and spleen emphasizes the systemic nature of the infection.

The clinical nature of the illness is related to the dose and virulence of the bedsonia (Gordon et al., 1957) and to the portal of entry. Little is known about the possibility of infection of man by the gastrointestinal tract. The bovine enteritis agent is excreted in the feces of cattle, and when it is fed to calves it induces illness, but the relation of this and the ornithosis and psittacosis agents, also excreted, to human disease is unexplored. It seems possible that the infector might be swallowed after hand to mouth transmission from the environment of an infected bird. As far as is known, human beings have not contracted psittacosis by ingestion of infected poultry. Bedsoniae are destroyed by heat, and poultry is ordinarily cooked long enough to destroy them. When concentrated highly infectious suspensions of the turkey ornithosis agent were spread on bread and given to mice they contracted generalized infections, most of them accompanied by pneumonia (Page, 1959a).

Poultry ornithosis can spread to any susceptible person in contact with an infected bird or its infective excretions. The probability of infection roughly parallels the extent of exposure, but high susceptibility, high virulence, or both may make even short exposure a risk.

The poultry flock in this country may be a small one raised by a farmer to supply his own family with food, or it may be an enormous commercial venture concerned with flocks numbering in the ten thousands. Those who may contract the infection from the flock are flockowners and their families and neighbors, anyone who visits the premises for any reason, flock caretakers, and anyone whom the flockowner may call in to ⟨...⟩ the flock when the illness becom ⟨...⟩ ive, such as feed company ⟨...⟩ products representatives, ⟨...⟩ spe-

AEROGENIC ROUTE OF INFECTION
OCCUPATIONAL-ACCIDENTAL

PSITTACOSIS

SINGLE AND HOUSE EPIDEMICS DUE TO CONTACT WITH INFECTED FEATHERS AND DUST.

SECONDARY HOSTS DUE TO CONTACT WITH INFECTED PSITTACINE BIRDS

MACAW

COCKATOO

COCKATIEL

CARITH
SASSHIN TRANSMISSION

PSITTACIDAE

PARROT

PARAKEET

FRINGILLIDAE

CANARY

BUTTERFLY FINCH

JAVA RICE BIRD

LEAF GOLD FINCH

ZEBRA FINCH

GEOPIZIDAE

LARIDAE
SEAGULL

SUSPECTED RESERVOIR HOSTS

FERRET

CANDIDESEMA

MAMMALIAN INFECTIONS WITH MEMBERS OF PSITTACIDE-LYMPHOGRANULOMA GROUP

NATURAL INFECTIONS:
SHEEP, CATTLE, CATS, OPOSSUM, MICE

ARTIFICIAL INFECTIONS:
MICE, GUINEA PIGS, HAMSTERS, GOPHERS, RABBITS, MONKEYS

IDENTIFICATION

FREQUENTLY MILD UNRECOGNIZED TO TYPHOID-LIKE INFECTIONS WITH FEVER, HEADACHE, PYELONEPHRIC INVOLVEMENT, NONPRODUCTIVE COUGH, ANOREXIA, PULSE SLOW, PROSTRATION AND TOXEMIA

SOURCE AND RESERVOIRS OF INFECTION

INFECTED AVIAN SPECIES

ONSET GROSS
CONTACT

RARELY SPUTUM FROM INFECTED PERSONS INCUBATION TIME: 7-15 DAYS NON-MAMMALIAN RESERVOIR-TRANSMISSION TO MAN NOT DETERMINED

DIAGNOSIS

ISOLATION OF VIRUS FROM BLOOD (1st -10th DAY) SPUTUM (SALIVA) AUTOPSY SPECIMENS SPLEEN, LIVER, EPICARDIUM

LARGE BASOPHILIC ELEMENTARY BODIES (L.C.L.)

SEROLOGIC TESTS

COMPLEMENT FIXATION TITER RISE IN PAIRED SERA DIRECT OR INDIRECT METHOD

TREATMENT

TETRACYCLINE DRUGS

ORNITHOSIS

CONTACT DURING RAISING AND DRESSING OF DOMESTIC FOWL

COLUMBIDAE
DOVE
PIGEON

ANATIDAE
GOOSE
DUCK
CHICKEN

MELEAGRIDAE
TURKEY

PHASIANIDAE
PHEASANT
GROUSE

PROCELLARIIDAE
FULMAR

THE RELATIVE IMPORTANCE OF AVIAN RESERVOIRS FOR HUMAN INFECTIONS UP TO 1955

PET BIRDS
PARRAKEETS
PARROTS
CANARIES

DOMESTIC BIRDS
TURKEYS
PIGEONS
DUCKS
CHICKENS

NON-DOMESTIC BIRDS
FULMARS
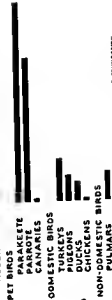
FIG. 23.12—Epidemology of psittacosis.

cialists, and veterinarians (Irons *et al.*, 1955; Osgood *et al.*, 1956; Schmidtke, 1957; Strauss *et al.*, 1960).

The next group to be exposed comprises all who participate, even indirectly, in preparation of infected birds for food. Anyone who removes the feathers and viscera from an infected bird is under some exposure as is anyone on the premises while this is being done. This includes housewives, cooks, butchers, all processing plant employees, especially killers, pickers, and eviscerators, but also plant superintendents and office workers, poultry inspectors, and anyone who removes the viscera from New York-dressed poultry. Certainly the processing of large flocks of infected birds provides ample opportunity for the bedsonia to spread to human beings. If the plant is not well ventilated, high concentrations of bedsoniae can build up. Undirected air currents may carry them throughout the plant. People who kill and defeather the birds are likely to come into contact with the droppings, and if the birds struggle and flop around, the bedsoniae are likely to be dispersed into the air (Ortel, 1961; Otto, 1962).

Still another group, exposed rather more at the periphery, is composed of those who dispose of infected dead birds or parts not used for food. This includes rendering plant employees and those who dispose of feathers and entrails and those who clean feces from eggs. In Oregon, 29 of 43 rendering plant employees became ill. It seems possible that unclean duck or goose feathers could carry the bedsonia; in some processing plants they are treated with heat. In Czechoslovakia, the age at which the infected ducks were slaughtered (4 weeks) suggests use of the feathers rather than use of the bird for food.

The recent poultry plant outbreaks have followed the seasonal marketing of large flocks for the holiday season in October to December or the culling of the flock after the egg-laying period, in April through June, particularly when flocks with unusual mortality are processed to protect the owner from further losses. The former seasonal incidence of psittacosis from psittacine cage pets, related to the Christmas exchange of birds and the closed winter household, should not mislead suspicion with respect to poultry. During May to August, 139 (4 per cent) of 3,150 members of families who kept ducks after they were taken from the incubator of a poultry farm contracted psittacosis (Serý and Strauss, 1957). Benign psittacosis was observed throughout the year among persons directly or indirectly exposed to pigeons which are usually bred all year (Mohr, 1954; Mumme, 1955; Kittel, 1955; Weyer and Lippelt, 1956). Steele and Scruggs (1958) called attention to the peak incidence from May through July but could find no satisfactory explanation for the increase at this season, apparent even when the occupational infections were excluded.

In the processing plant outbreaks there has been no noticeable difference in the susceptibility of men and women. Exposure has been a more critical factor than either age or sex.

Laboratory Infections

The incidence of laboratory infections bears emphasis. During the height of the experimental investigations after the epidemics of 1929 and 1930, 38 laboratory infections were contracted and 5 of the infected died. In the United States from 1931 to 1956, 70 laboratory-acquired infections with 7 deaths remind scientific personnel and animal caretakers that utmost care must be taken in handling specimens suspected of carrying bedsoniae and in carrying out the diagnostic procedures (Moltke, 1932; Sulkin and Pike, 1949; Meyer and Eddie, 1958a). At the Statens Serum Institut in Copenhagen, among a staff of 5, 4 contracted psittacosis—a charwoman, a technician and 2 physicians. One of these physicians contracted the disease three times (Christensen, 1957a). Eight of the laboratory personnel working on the Texas outbreak became infected, some of them repeatedly (Davis and Delaplane,

1955), and workers handling turkey bedsoniae from Oregon and New Jersey have also become infected.

The case fatality rate for the outbreak of psittacosis due to contact with psittacine birds in 1892 was 35 per cent in France, and in 1929 and 1930 involving many countries, nearly 20 per cent. In some outbreaks it was 40 to 100 per cent, but with the recognition of mild infections the rate was about 10 per cent. Then with the introduction of antimicrobial drugs it has fallen to 5 per cent in the United States and to 3.5 per cent (86 cases) in Germany (Haussman et al., 1956) and on down to 0.5 per cent in 550 cases during 1955 to 1957 (Reinwein and Walther, 1961). Most of the patients who died were 40 to 60 years old. A recent analysis of the Faeroe Island outbreaks due to contact with infected petrels corrects previous statistics. It revealed that in the over-all reported mortality of 22 per cent (182 cases with 40 deaths), there existed a noteworthy difference in the fatality rates of nonpregnant and pregnant women. In men and nonpregnant women the rate was 16.2 per cent (154 with 25 deaths) but in 14 pregnant women with 11 deaths, it rose to 78 per cent (Vaag, 1959).

Under the title, "Domesticated pigeons do not carry or spread disease to human beings," the author of "The Pigeon," Wendell M. Levi (1960), repeats that pigeons are not potentially dangerous as spreaders of psittacosis, that the human fatality of 1 per cent misrepresents the case, and that children under 10 years old cannot catch psittacosis. Present knowledge contradicts these statements: (1) All recent studies and reviews, not all officially recorded, support the view that the pigeon is a noteworthy reservoir of ornithosis throughout the world, causing clinical and subclinical human infections (Jansson, 1960) (2) In the 164 cases of psittacosis proved epidemiologically and in some instances through isolation of a bedsonia of low virulence, 8 patients (4.8 per cent) died; some patients died even though they had been treated with antimicrobial drugs. (3) Infants and children may die of psittacosis (Berman

et al., 1955; Ephrati-Elizur and Bernkopf, 1956); in fact, the case fatality rate in 24 cases epidemiologically assumed to be due to contact with pigeons was 33 per cent.

The mortality in poultry–particularly turkey–processing plant employees has certainly not been negligible: 2 of 22 in one outbreak, 4 of 44 in another (Irons et al., 1955), 2 of 86 in another (Osgood et al, 1956), and 1 of 41 in two plants in Texas (Leachman and Yow, 1958). The deaths occurred in cases not clinically recognized as psittacosis; broad-spectrum antibiotic therapy either was not instituted at all or was much delayed, or the injury to the liver and kidney was by then so extensive that treatment was ineffectual (Yow et al., 1959). Despite the low death rate of 1.3 per cent in 150 cases observed during some outbreaks due to contact with ducks, ornithosis is a serious disease; the convalescence may be long and tedious. Even after a year of convalescence, working capacity may be restricted and it may lead to severe involvement of the cardiopulmonary system (Strauss, 1957).

PROPHYLAXIS, SUPPRESSIVE AND CONTROL MEASURES

Prophylaxis and control of ornithosis in poultry must take into consideration (1) prevention of epizootics and spread of infection among poultry, (2) protection of the flockowner and workers in processing and rendering plants, and (3) the consumer. In the United States, the official steps taken can be applied to poultry in general, though they were adopted as measures against occupational human psittacosis due to diseased turkeys. German veterinarians recommend that ornithosis in pigeons be suppressed by reducing the feral pigeon population in cities and by treating racing pigeons with antimicrobial drugs at regular intervals (Monreal, 1963).

Measures to Prevent Ornithosis on Poultry Farms

The United States Department of Agriculture, according to a regulation passed on April 9, 1957, prohibits the movement

of poultry, carcasses, and offal from premises where the existence of ornithosis has been proved by isolation of the bedsonia. The Agricultural Research Service, U.S. Department of Agriculture, and the Food and Drug Administration forbid movement of all birds from infected flocks in interstate commerce.

Until the means by which this infection spreads has been learned, the flockowner can only apply the general principles of control of infectious diseases. He should be informed about this disease through agricultural authorities and trade journals. If he finds evidence of disease during daily observation of his flock, he should at once isolate any sick birds and submit dead birds to a poultry laboratory. This is very important because sick birds excrete bedsoniae and the environment can become hopelessly contaminated as an epizootic progresses. Early isolation of the sick birds might go a long way toward checking its spread throughout a flock.

The acquisition of infected poultry is a means of introducing the disease into a flock. All breeders should make every effort to acquire any new stock from flocks known to be free from this infection.

Immunization has not been considered warranted. It would be necessary first to develop an effective immunizing preparation, a potentially difficult research problem, and then to solve the problems, technical and economic, of commercial production. This would be a major undertaking, and it is questionable whether a vaccine could be produced at a cost low enough to permit its routine use. The outlook is not very promising.

Once the infection has been diagnosed another difficult control problem is apparent. Treatment with antibiotics in the feed or water does reduce morbidity and mortality, but it is expensive and, with the dosage and treatment schedule recommended, of uncertain effectiveness. For the time being, if virulent ornithosis has been proved to exist on a farm it is imperative to depopulate the entire premises of birds in a manner to be decided by the agricultural disease control agency in consulta-

tion with the local or state health department. Dead birds should be disposed of in some way that insures that the bedsoniae will not be spread, either to other birds or to man. The methods have been described by Bodenweiser and Peterson (1958). Eggs from infected flocks should be thoroughly disinfected and hatched in separate incubators. There are no restrictions of the movement of eggs from infected flocks. Hatcherymen have refused to handle eggs from farms where the infection may be widespread; no assurance can be furnished that they are not unknowingly handling eggs from infected flocks. Poults should be under observation and supervision by the poultry division of the state department of agriculture. All poultry quarters should be thoroughly disinfected with hot lye solution after the disposal of the infected flock. The cost to the flockowner and the cost of continuous supervision make it seem unlikely that ornithosis-free flocks can be established.

The poultry industry should stimulate greater interest in the specific diagnosis of poultry diseases, and it may be found necessary for more diagnostic laboratories to include tests for ornithosis. This already seems warranted. Any excess of serum samples collected for *Salmonella pullorum* tests could be used. The existence of ornithosis can be readily established through serum tests and examination of a few sick birds. Although neither the clinical course nor the gross lesions are characteristic enough to allow diagnosis without bedsonia isolation, or at least serologic tests, the existence of a diarrheal or respiratory infection causing oxidation on organs of the peritoneal cavity should provoke suspicion. Agricultural agencies should insist that poultry inspection services report any suspicious lesions observed on the processing line (Meyer, 1955).

Protective Measures Against Human Infections in Processing Plants

Any poultry diagnostic laboratory that recognizes ornithosis in poultry should at once notify public health authorities so that they can advise physicians in the

area of the possibility of human infection. The flockowner must be informed that he, his family, flock caretakers, and anyone who comes on the premises may be under exposure and at risk of infection. The symptoms of the oncoming disease should be described so that medical attention will be sought early in the course. Care of a flock undergoing a severe epizootic requires extra work, and this may lower the resistance of the caretakers.

Unfortunately the processing plant group has been the first point at which the infection in the flocks has been recognized, especially in the turkey outbreaks. Ideally, if the infection had already been diagnosed in the flock this group would never come into contact with the bedsonia. When flocks with high infection rates caused by highly infective bedsonia are processed, the incidence of disease and latent infection among processing plant personnel will be high. The plants can be expected to refuse to process flocks in which the infection is suspected rather than subject plant personnel to the risk. If the plant were to send out inspectors to observe the flock before it is moved into the plant, the inspector might notice signs of the infection such as droopiness of the birds and evidence of extensive diarrhea in yellowish droppings. Although this alone cannot be relied on to reveal the infection, it is a necessary part of the inspection. General sanitation and ventilation in the plant might lower the risk, but exposure in certain phases of the processing is likely to be extensive. If the flock is to be processed despite the suspicion of infection, the personnel must take pains to minimize distribution of the infective particles adherent to the wings and in the organs, but particularly the exudates and feces should be regarded as highly infections (Meyer, 1964).

The poultry meat inspector must promptly notify his superior and the plant manager when he encounters gross lesions suggestive of ornithosis. Laboratory tests must decide whether the lesions are caused by bedsonia or other agents.

Unfortunately, most of the processors have already been exposed when the diagnosis is made. Then the personnel must be informed of the possibilities and all physicians in the area must be notified. Now that effective treatment is possible, serious illness or death can be prevented in most cases if treatment is given early enough. The problem has been that the infection has not been recognized, sometimes not until after several people have died of psittacosis pneumonitis.

In districts where turkey ornithosis annually appears as latent epizootics of varying intensity, contact with grossly diseased carcasses passing through processing plants where the birds are dressed is unavoidable. Employees of such establishments should be under constant public health and occupational hygiene supervision; serologic tests should be made at frequent intervals. If they have been exposed to diseased birds they should remain under medical supervision and care. In case they contract psittacosis they must be treated thoroughly with antibiotic drugs.

To protect rendering plant employees, such as truck drivers and plant workers, one problem is to prevent formation of heavily infected aerosols during plant operations. The bedsonia is destroyed within 3½ to 5 minutes at 132.8° F. (56° C.), so that after proper cooking the byproducts can be considered safe. Rendering plants should install any special equipment needed, the plants should be well ventilated, and the ventilation system should be constructed so that they constitute no risk (see Oregon State Board of Health: Joint Statement of Policy, etc., 1956).

Protection of home processors is considerably more complicated, but this group should be observed, especially if processing plants reject flocks and unscrupulous poultry raisers are left to dispose of their flocks in any way possible.

Protection of the Consumer

So far as is known the infection has not been contracted through ingestion. The danger of infection in market poultry is in the eviscerating, and it has been con-

tracted in the dressing of even frozen birds (Bowmer, 1958). The U.S. Department of Agriculture Regulations Governing the Inspection of Poultry and Poultry Products (1961) recommended that the sale of birds from which the viscera have not been removed be prohibited, and if this recommendation were followed, the person who prepares the birds for the table would be protected. The finding of antibodies against the group antigen in people who have had no known contact with psittacine birds suggests that poultry dressing may have introduced the infection and that it went undiagnosed. It is fairly certain that ornithosis has been prevalent on pigeon and duck farms for some time, and doubtless thousands of infected birds have been distributed through commercial channels without recognized harm to the consumer.

Early public actions prompted by outbreaks of psittacosis among the personnel on breeding and fattening farms of water fowl, particularly ducks and geese, mainly in eastern Europe, devoted considerable speculation to preventive measures (Kielstein, 1961). After the war, keeping and fattening of ducks became a profitable undertaking since a breed of ducks was developed that, under optimal conditions of feeding, was ready for consumption within 8 to 9 weeks. The rapidly growing industry presented many new problems and risks to the operators and employees of processing plants. Since the early investigators were epidemiologists and public health workers their interest focussed on prevention of the workers; they unanimously agreed that the establishment of ornithosis-free duck breeding and fattening farms would eliminate human disease. Subsequent investigations by veterinarians showed that this would be difficult because of the complexities of the already known and the many unknown factors on the farms. According to the latest reports of experts, the hygienic measures must take into consideration the following: proper intensive and diversified feeding, avoidance of crowding of the birds on stagnant polluted water, installation of running water, drainage through concrete-lined interchannels, and repeated serologic examination of duck blood samples in order to gauge the extent of the ornithosis in the flocks. These measures may check the progressive epizootization of the duck population and in turn reduce the number of infected birds that reach the poultry processing plant. Heavily infected flocks should be promptly slaughtered and processed under veterinary and medical supervision.

Complete eradication of ornithosis is not anticipated in the near future. Every reasonable means, however, should be adopted to keep the infection rate of the flocks low. Attention must be paid to ducklings infected in the egg. When visibly sick, they must be isolated. The risk of infection to duck flocks through contact with wild birds is considered minimal (Voigt et al., 1962). To reduce the build-up of the bedsonia infection within the duck population and their environment, Wolff (1961) recommended chemotherapy and prophylaxis similar to that used in the U.S.A. on turkey farms. The experiences with attempts to free pigeon flocks from ornithosis (Meyer and Eddie, 1955; Shipkowitz et al., 1958) should warn the proponents that this type of prophylactic measure, even if partially effective, is economically and administratively impractical. All control measures must adapt themselves to the prevailing epizootic, regional, and economic situations.

Until the measures outlined have been tested and proved effective, the German physicians and veterinarians insist that no efforts be spared to protect the poultry farmers, their families, and the workers in the poultry plants by breaking the transmission chain from ducks and geese to man. Efforts should be made to recognize the epidemic and sporadic human cases through constant medical supervision and prolonged chemotherapy (Grantova and Milek, 1962). Of special interest are the following actions taken: Regulations have been promulgated to enforce prolonged scalding of the carcasses at temperatures

above 60° C. (140° F.) (Grahneis and Horn, 1961), mechanical plucking of feathers, and evisceration in order to reduce infective aerosols. This procedure does not prevent contact or "smear" infections. Continuous disinfection within the processing plant and proper spacing of the employees on the working benches in the installations is considered important. Since personnel continuously exposed to bedsonia may acquire some resistance it is recommended that inexperienced susceptible housewife brigades should not be drafted in emergencies for service in poultry processing plants. To minimize concentration of the infective agent the flock should be observed antemortem and any visibly sick birds

eliminated. Distant transportation and holding of the birds without food and water in crowded cages for long periods before slaughter, particularly during July to October when ornithosis is prevalent, should be avoided. Combinations of environmental stresses contribute to the conversion of latent to active infection with heavy shedding and dispensing of the infective agent within and without the bird delivered to the plants (Wolff, 1961; Thamm, 1964).

Early recognition of ornithosis in poultry, in particular turkey and duck farms, is essential and basic in providing effective hygienic and prophylactic measures to prevent losses and human infections.

REFERENCES

Allen, E. G., and Bovarnick, M. R.: 1957. Association of reduced diphosphopyridine nucleotide cytochrome C reductase activity with meningopneumonitis virus. Jour. Exper. Med. 105:539.

———, and Bovarnick, M. R.: 1962. Enzymatic activity associated with meningopneumonitis. Ann. N.Y. Acad. Sci. 98:229.

———, Girardi, A. J., Sigel, M. M., and Klein, M.: 1953. Studies on the psittacosis-lymphogranuloma group. III. The effect of aureomycin on the propagation of virus in the chick embryo. Jour. Exper. Med. 97:783.

———, Kaneda, B., Girardi, A. J., Scott, T. F. McN., and Sigel, N. M.: 1952. Preservation of viruses of the psittacosis-lymphogranuloma venereum group and herpes simplex under various conditions of storage. Jour. Bact. 63:569.

———, Ostroff, G., and Bovarnick, M. R.: 1960. Inhibition of the cytochrome reductase activity associated with meningopneumonitis virus by fatty acids derived from infected allantoic fluids. Virology 11:737.

Allison, A. C., and Perkins, H. R.: 1960. Presence of cell walls like those of bacteria in Rickettsiae. Nature 188:796

Andrewes, C. H., and Mills, K. C.: 1943. Psittacosis (ornithosis) virus in English pigeons. Lancet 244:i:292.

Andrews, J. M.: 1957. The importance of psittacosis in the United States. Jour. Am. Vet. Med. Assn. 130:109.

Andrianne, V. F.: 1952 Ornithosis among pigeons in Belgium. Rec. Med. Vet. 96:523.

Armstrong, J. A., Valentine, R. C., and Fildes, C.: 1963. Structure and replication of the trachoma agent in cell cultures, as shown by electron microscopy. Jour. Gen. Microbiol. 30.59.

Arnstein, P: 1963. Unpublished data. Drug sensitivity of bedsonia isolated from organs of parrots treated for ornithosis by aureomycin.

———, Cohen, D. H., and Meyer, K. F.: 1964. Medication of pigeons with chlortetracycline in feed. Jour. Am Vet Med Assn. 145:921.

Austin, F. J.: 1957. Quoted in Foreign Letters, Jour. Am. Med. Assn. 164:1607 (Proc. Univ. Otago Med. School 35-7, 1957).

Babudieri, B.: 1956. L'ornitosi, sua presenza e frequenza in Italia. Terapia 41:3.

———, and Cerri, R.: 1956. Osservazione di casi umani di ornitosi a Genova. Rend. Ist. Sup. San., Roma 19:59.

———, and Moscovici, C.: 1955. Lesioni istopatologiche del polmone del topino inoculato con un virus ornitosico di scarsa virulenza. Rend. Ist. Sup. di San. Roma 18:446.

———, and Secchi, P.: 1952. La reazione di agglutinazione nella aerodiagnosi dell'infezione da Coxiella burneti. Rend Ist. Sup. San, Roma 15 584.

Bacon, A. P. C.: 1953. Psittacosis. Two farm cases. Lancet 265 (Aug. 22) 2.376.

Bader, J. P., and Morgan, H. R.: 1958. Latent viral infection of cells in tissue culture. VI. Role of amino acids, glutamine, and glucose in psittacosis virus propagation in L cells. Jour. Exper. Med. 108.617.

———, and Morgan, H. R.: 1961. Latent viral infection of cells in tissue culture. VII. Role of water-soluble vitamins in psittacosis virus propagation in L cells. Jour. Exper. Med. 113:271.

Barwell, C. F.: 1952. Psittacosis-lymphogranuloma infection. Med. Illust. 6:281.

———: 1952. *Some observations on antigenic structure of psittacosis and lymphogranuloma venereum viruses; treatment of virus suspension by various reagents and specific activity of acid extracts.* Jour. Hyg., Camb. 53:263.

———: 1957. Psittacosis. Practitioner, London 179:500.

———, and Bishop, L. W. J.: 1951. Virus of enzootic abortion in ewes: antigenic relationship with viruses of the psittacosis group. Nature, London 167:998.

Basova, N. N., and Levi, N. I.: 1960. The complement-fixation inhibition test. Prob. Virol. 5.281.

———, Suchkov, Y. G., Gusev, V. M., and Rudnev, M. M.: 1960. Ornithosis in wild and domestic birds. Jour. Microbiol., Epidemiol. and Immunobiol., London 31:1577.

Beasley, J. N., Davis, D. E., and Grumbles, L. C.: 1959 Preliminary studies on the histopathology of experimental ornithosis in turkeys. Am. Jour. Vet. Res. 20,541.

———, Moore, R. W., and Watkins, J. R.: 1961. *The histopathologic characteristics of diseases producing inflammation of the air sacs in turkeys. A comparative study of pleuropneumonia-like organisms and ornithosis in pure and mixed infections.* Am. Jour. Vet. Res. 22:85.

Beaudette, F. R., Hudson, C. B., and Kaschula, V. R.: 1956. An outbreak of psittacosis. Vet. Med. 51:496.

Bedson, S. P.: 1932. The nature of elementary bodies in psittacosis. Brit. Jour. Exper. Path. 13.65.

———: 1935. Use of complement-fixation reaction in diagnosis of human psittacosis. Lancet 229:2:1277.

———: 1937. Observations on the complement-fixation test in psittacosis. Lancet 233:2:1477.

———: 1938. A study of experimental immunity to the virus of psittacosis in the mouse, with special reference to persistence of the infection. Brit. Jour. Exper. Path. 19:353.

———: 1959. *The Harben Lectures, 1958. The psittacosis-lymphogranuloma group of infective agents. Lecture I: The history and the characters of these agents* Jour. Roy. Inst. Pub. Health and Hyg. 22.67.

———: 1959a. *The Harben Lectures, 1958. The psittacosis lymphogranuloma group of infective agents. Lecture II: The position of the agents of trachoma, inclusion conjunctivitis and cat-scratch disease. The ecology of the agents of the psittacosis-lymphogranuloma group.* Jour. Roy. Inst. Pub. Health and Hyg. 22.99.

———: 1959b. *The Harben Lectures, 1958. The psittacosis-lymphogranuloma group of infective agents. Lecture III: The diagnosis, treatment and control of infection of man with these agents. The taxonomic position of the group.* Jour. Roy. Inst. Pub. Health and Hyg. 22:151.

———, Barwell, C. F., King, E. J., and Bishop, L. W. J.: 1949. Laboratory diagnosis of lymphogranuloma venereum. Jour. Clin. Path. 2:241.

———, and Bland, J. O. W.: 1932. Morphological study of psittacosis virus, with description of the developmental cycle. Brit. Jour. Exper. Path. 13:461.

———, and Bland, J. O. W.: 1934. The developmental forms of psittacosis virus Brit. Jour. Exper. Path. 15:243.

———, and Gostling, F. V. T.: 1954. A study of the mode of multiplication of psittacosis virus. Brit. Jour. Exper. Path. 35:299.

———, and Western, G. T.: 1930. A disease of parrots communicable to man (psittacosis). Aetiology—experimental observations. Rep. Pub. Health and Med. Subj., no. 61, pp. 59–107.

Beech, M. D., and Miles, J. A. R.: 1953. Psittacosis among birds in South Australia. 1. A survey of infection in some common species in 1951 and 1952. Australian Jour. Biol. and Med. Sci. 31:423.

Benedict, A. A.: 1958a Intradermal test for detection of ornithosis exposure in chickens and turkeys. *In: Progress in Psittacosis Research and Control*, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 127–38.

———: 1958b. Growth of meningopneumonitis virus in normal and immune guinea pig monocytes. Nature 181:1742.

———, and McFarland, C.: 1956a. Direct complement fixation test for diagnosis of ornithosis in turkeys. Proc. Soc. Exper. Biol. and Med. 92:768.

———, and McFarland, C.: 1956b. Antigenic studies on the psittacosis-lymphogranuloma venereum group of viruses. IV. Studies on cutaneous hypersensitivity in chickens infected with ornithosis virus. Jour. Immunol. 77:165.

———, and McFarland, C.: 1958. Newer methods for detection of avian ornithosis. Ann. N.Y. Acad. Sci. 70:501.

———, and O'Brien, E.: 1956. Antigenic studies on the psittacosis lymphogranuloma venereum group of viruses. II. Characterization of complement-fixing antigens extracted with sodium lauryl sulfate. Jour. Immunol. 76.293.

———, and O'Brien, E.: 1958. A passive hemagglutination reaction for psittacosis. Jour. Immunol. 80.94.

———, O'Brien, E., and McFarland, C.: 1956. Antigenic studies on the psittacosis-lymphogranuloma venereum group of viruses. III. Detection of ornithosis hypersensitivity in experimentally infected chickens. Am. Jour. Vet. Res. 17:543.

Benedict, A. A., Tips, R. L., and Eddy, D.: 1955. Antigenic studies on the psittacosis-lymphogranuloma group of viruses. I. Acid-soluble complement-fixing and skin test antigens. Texas Rep. Biol. and Med. 13.206.

Benson, H. N., Brumfield, H. P., and Pomeroy, B. S.: 1961. Requirement of avian C_1 for fixation of guinea pig complement by avian antibody-antigen complexes. Jour. Immunol. 87.616.

Berendt, R. F., Beard, C. W., and Brown, E. M.: 1962. Studies on aerosol-induced psittacosis in rhesus monkeys. U.S. Army Chem. Corps Biol. Lab., Fort Detrick, Tech. Memo. 6. 19 pp.

Berman, S. Freundlich, E., Glaser, K. Abrahamov, A., Ephrati-Elizur, E., and Bernkopf, H.: 1955. Ornithosis in infancy. Pediatrics 15.752.

Bernkopf, H.: 1952. Trachoma virus—recent developments. Prog. Med. Virol. 4.119.

⸺, Nishmi, M., Maythar, B., and Feitelberg, L.: 1960. Darkfield agglutination of fluoro carbon-treated trachoma virus by serums of trachoma patients and immunized rabbits. Jour. Infect. Dis. 106.83.

Besson, A.: 1958. L'ornithose ou maladie transmise par les pigeons. Bul. Acad. Nat. Méd. 142:81.

Bezdenchnykh, I. S.: 1960. (Sanitary and hygienic measures for prevention of ornithosis.) Gig. Sanit. 25:101.

Bland, J. O. W., and Canti, R. G.: 1935. Growth and development of psittacosis virus in tissue cultures. Jour. Path. and Bact. 40:231.

Bodenweiser, L. E., and Peterson, K. J.: 1958. Successful method of destroying large numbers of turkeys under field conditions. Jour. Am. Vet. Med. Assn. 132.238.

Boldyrev, S. T.: 1961. Detection of complement-fixing antibodies to ornithosis virus in the blood of the cormorant (Phalacrocorax carbo sinensis) Prob. Virol. 6.538.

Bolotovskii, V. M.: 1959. The resistance of ornithosis-psittacosis virus to the action of physical and chemical agents. Prob. Virol. 4.63.

Boney, W. A., Jr., Grumbles, L. C., Delaplane, J. P., Irons, J. V., and Sullivan, T. D.: 1952. Another agent causing air sac involvement in turkeys. Jour. Am. Vet. Med. Assn. 121:289.

Bouchet, H., and Stutter, V.: 1951. A propos de treize cas d'ornithose. Lyon Méd. 185:381.

Boulanger, P., and Ruce, C. E.: 1954 A study of complement fixation methods as applied to the demonstration of antibodies in birds. Proc. 90th Ann. Meet. Amer. Vet. Med. Assn. 1953 (1954), pp. 316–21.

Bowmer, E. J.: 1958. A human outbreak of psittacosis due to infected turkeys in a poultry-processing plant. Canad. Jour. Pub. Health 49:27.

Brand, G., and Lippelt, H.: 1954. Zur Herstellung von Antigenen für die Ornithose (Psittakose)-Komplementbindungsreaktion. Zeitschr. f. Hyg. u. Infektionskr. 140:175.

Brumfield, H. P., Benson, H., and Pomeroy, B. S.: 1961. Procedure for modified complement fixation test with turkey, duck and chicken serum antibody. Avian Dis. 5.270.

⸺, and Pomeroy, B.S.: 1957. Direct complement fixation by turkey and chicken serum in viral systems. Proc. Soc. Exper. Biol. and Med. 94:146.

Bucca, M A.: 1958. Comparison of CF and HI tests on psittacosis-LGV serums. Pub. Health Rep. 73:461.

Buckley, S. M., Whitney, E., and Rapp, F.: 1955. Identification by fluorescent antibody of developmental forms of psittacosis virus in tissue culture. Proc. Soc. Exper. Biol. and Med. 90.226.

Burnet, F. M.: 1935. Enzootic psittacosis amongst wild Australian parrots. Jour. Hyg. 35:412.

⸺: 1939. A note on the occurrence of latal psittacosis in parrots living in the wild state. Med. Jour. Australia 1:545.

⸺, and Rountree, P. M.: 1935. Psittacosis in the developing egg. Jour. Path. and Bact. 40 471.

Busila, V. T., Alexandrescu, R., and Bacaloglu, D.: 1960 (Clinical and pathomorphological aspects of an epidemic produced by the ornithosis virus) Stud. Cercet. Inframicrobiol. 11:187.

Buttitta, P. I., and Nobili, I.: 1953. Indagini sull'ornitosi in Sicilia. Clin. vet. Milano 76:369.

Carlson, H. C., Whenham, G. R., and Bigland, C. H.: 1961. Ornithosis in turkeys in Alberta. Avian Dis. 5:51.

Carski, T. R.: 1961. The use and limitations of the fluorescent antibody technic in the identification and localization of viruses. Am. Jour. Clin. Path. 35:260.

Chang, L., San pin, W., and Grayston, J. T.: 1962. Antigenic relationships of trachoma virus strains in mouse toxicity prevention tests. Ann. N.Y. Acad. Sci. 98:347.

Chervonskii, V.: 1960. Detection of complement-fixing antibodies in certain species of birds and mammals in the complement-fixation test with ornithosis virus antigen. Prob. Virol. 5.85

⸺, and Popova, A. M.: 1959. An antigen for the ornithosis-psittacosis complement-fixation test. Prob. Virol. 4.67.

Christensen, P.: 1957a. Ornithosis. A study of virus and antigen. Copenhagen: S L. Møllers Bogtrykeril, 110 pp. (Thesis)

⸺: 1957b. Ornithosis. A study of virus and antigen. Acta Path. et Microbiol Scandinav. (suppl) 118, p. 110.

Cohen, L., Gray, I., and London, S.: 1946. Pneumonia of ornithotic origin. Report of two cases occurring in the same family following contact with pigeons. New York State Jour. Med. 46.1132.

Coles, J. D. W. A.: 1940. Psittacosis in domestic pigeons. Onderstepoort Jour. Vet. Sci. 15:141.

Colón, J. I.: 1960. Enzymes for formation of citrovorum factor in members of the psittacosis group of microorganisms. Jour. Bact. 79.741.

——: 1962. The role of folic acid in the metabolism of members of the psittacosis group of microorganisms. Ann. N.Y. Acad. Sci. 98:234.

——, and Moulder, J. W.: 1958. Folic acid in purified preparations of members of the psittacosis group of microorganisms. Jour. Infect. Dis. 103:109.

Cook, L.: 1962. Ornithosis in feral pigeons. Australian Jour Exper. Biol. Med. Sci. 40:389.

Cox, H. R.: 1955. Chemotherapy of psittacosis. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 137–54.

Crocker, T. T.: 1954. The number of elementary bodies per 50% lethal dose of meningopneumonitis virus as determined by electron microscopic counting. Jour. Immunol. 73:1.

Dahmen and Hamet: 1930. Quoted by Eikeles, C. and Gryzmer, B.: 1932. Die Psitacose unter besonderer Berucksichtigung der Veterinären Dufferentialdiagnose. Tierärztl. Rundschau. 38 847.

Dane, D. S.: 1955. Some observations on the complement-fixation test for the psittacosislymphogranuloma venereum group of viruses. Med. Jour. Australia 1:382.

——, and Beech, M.: 1955. Psittacosis among birds in contact with man. Med. Jour. Australia 1:428.

Davis, D. E.: 1955. Some lesions of turkey ornithosis. Southwest. Vet. 9:128.

——, and Delaplane, J. P.: 1955. Ornithosis in turkeys. Proc. Book, 92nd Ann. Meet., Am. Vet. Med. Assn., p. 296.

——, and Delaplane, J. P.: 1958a. The lesions of ornithosis in turkeys. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 89–110.

——, and Delaplane, J. P.: 1958b. Turkey ornithosis prophylaxis using tetracycline. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 204–12.

——, Delaplane, J. P., and Watkins, J. R.: 1957a. The role of turkey eggs in the transmission of ornithosis. Am. Jour. Vet. Res. 18:409.

——, and Watkins, J. R.: 1959. The effect of chlortetracycline on the immunological response of turkeys infected with ornithosis. Jour. Infect. Dis 104.56.

——, Watkins, J. R., and Beasley, J. N.: 1958. Experimental psittacosis and ornithosis in turkeys. Comparison of nine strains. Avian Dis. 2:315.

——, Watkins, J. R., and Delaplane, J. P.: 1957b. The treatment of turkey ornithosis in a farm flock. Southwest. Vet. 10:223.

Davis, D. J.: 1948. Complement fixation reactions in psittacosis. Arch. Int. Med. 81.621.

——: 1949. The use of phenolized allantoic fluid antigen in the complement fixation test for psittacosis. Jour. Immunol 62:193.

——: 1950 Psittacosis infections in feral pigeons. Jour. Am. Vet. Med. Assn. 116.220.

——: 1955 Psittacosis in pigeons. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp 66–73.

——, and Ewing, C. L.: 1947. Recovery of ornithosis virus from pigeons in Baltimore, Maryland. Pub. Health Rep. 62:1484.

——, and Vogel, J. E.: 1949. Recovery of psittacosis virus from chicks hatched from inoculated eggs. Proc. Soc. Exper. Biol. and Med. 70.585

de Burgh, P., Jackson, A. V., and Williams, S. E.: 1945. Spontaneous infection of laboratory mice with a psittacosis-like organism. Australian Jour Exper. Biol. and Med. Sci. 23:1007.

Dekking, F.: 1949 Postduiven en psittacosis. Nederl. Tijdschr. v. Geneesk. 93:4338.

——: 1950. Psittacosis en Ornithosis in Nederland. Thesis, Amsterdam.

——: 1961. Psittacosis-ornithosis van ornithosis. Tijdschr. Diergeneesk. 86 62.

——: 1963. Epidemiology of ornithosis and psittacosis. Arch. Ges. Virusforsch. 13:316.

——, and Duyic C.: 1954. Psittacose et ornithose en Hollande. Rev Belge Path. et Méd Exper 21:92.

Delaplane, J. P.: 1958. Ornithosis in domestic fowl: newer findings in turkeys. Ann. N Y Acad. Sci. 70 495.

Derzsy, D.: 1958. (Ornithosis cases in chicken farms) Magyar Allator. Lap. 12.172. (English summary)

Dew, J., Mawson, K., Ellman, P., and Brough, D.: 1960. Ornithosis in two railway guards, an occupational hazard. Lancet 2:18.

Deyke, V. F., and Meyer, J. R.: 1965. Psittacosis. A report of two cases occurring in pigeon breeders. Nebraska State Med. Jour. 40 203.

Dickerson, M. S.: 1962. Turkey ornithosis in man. Texas outbreaks. Texas State Jour. Med. 58.916.

Dickinson, E. M., Babcock, W. E., and Kilian, J. G.: 1957. Ornithosis in Oregon turkeys. Jour. Am. Vet. Med. Assn. 130:117.

Domok, I.: 1963. Ornithosis epidemics of the last two years in Hungary. Arch. Ges. Virusforsch. 13.323.

Donaldson, P., Davis, D. E., Watkins, J. R., and Sulkin, S. E.: 1958. The isolation and identification of ornithosis infection in turkeys by tissue culture and immunocytochemical staining. Am. Jour. Vet. Res. 19.958.

Do Valle, L. A. R.: 1950. Infecçoes do grupo psitacose em psitacídeos e colombídeos brasileiros. Isolamente de vírus. V. Cong. Internat. Microbiol. Rio de Janeiro, p. 104.

Drobyshevskaya, A. I., Figarevsky, V. E., and Smorodintsev, A. A.: 1962. Activity of phagocytic factors in experimental infection of white mice with mouse pneumonia and meningopneumonia viruses. Acta Virol. 6.158.

Duncan, P. R., Thomas, A. E., and Tobin, J. O'H.: 1952. Ornithosis. Isolation of virus from a case. Lancet 262:696.

Early, R. L., and Morgan, H. R.: 1946a. Studies on the chemotherapy of viruses in the psittacosis-lymphogranuloma venereum group. III. Effect of certain chemotherapeutic agents on the growth of psittacosis (6BC strain) in tissue cultures and eggs. Jour. Immunol. 53:151.

——, and Morgan, H. R.: 1946b. Studies on the chemotherapy of viruses in the psittacosis-lymphogranuloma venereum group IV. Effect of certain chemotherapeutic agents on psittacosis virus (6BC strain) infections in mice. Jour. Immunol. 53:251.

Eaton, M D.: 1945. Serological differentiation of primary atypical pneumonia from the virus pneumonia of the psittacosis group. Proc. Soc. Exper. Biol. and Med. 60:231.

——: 1950 Chemotherapy of virus and rickettsial infections Ann. Rev. Microbiol. 4:223.

Eddie, B., and Francis, T., Jr.: 1942. Occurrence of psittacosis like infection in domestic and game birds of Michigan. Proc. Soc. Exper. Biol. and Med 50 291.

——, Meyer, K. F., Lambrecht, F. L., and Furman, D. P.: 1962. Isolation of ornithosis bedsoniae from mites collected in turkey quarters and from chicken lice. Jour. Infect. Dis. 110:231.

Eilenberger, B. K., and Miller, C. M.: 1952. Psittacosis in a family. Brit. Med. Jour. 2:189.

Ephrati-Elizur, E., and Bernkopf, H.: 1956. Isolation of six strains of ornithosis virus from children with infections of the respiratory tract. Jour. Infect. Dis. 98:45.

Evans, C. A., and Moore, G. E.: 1950 The effects of viruses on intracocular tissues. II. Infections with the viruses of herpes simplex, feline agranulocytosis and ornithosis. Jour. Infect. Dis. 87:1.

Fagan, R.: 1958. Direct comparison of chick embryo and mouse in the isolation of the psittacosis agent. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp 111–16.

Fallot, G H.: 1951. L'Ornithose. Variété nouvelles de pneumonie atypique, Masson et Cie, Paris, 129 pp.

Findlay, G. M., MacKenzie, R. D., and MacCallum, F O.: 1938. Morphological study of virus of lymphogranuloma inguinale (climate bubo). Trans. Roy. Soc Trop. Med. and Hyg. 32:183.

Fischer, R. R.: 1955. Etwas über den Stand der Psittakosis in Westdeutschland, diesmal in anderer Schau Medizinische (Stuttgart), p 1038

Fitz, R. H., Meiklejohn, G., and Baum, M. D.: 1955 Psittacosis in Colorado. Ann. Jour. Med. Sci. 229:252.

Fortner, J.: 1958. Sur la question de l'immunité contre la psittacose. Bul. Office Internat. d'Hyg. Pub. 28 683.

Francis, D. W.' 1960. Case report—an outbreak of ornithosis in New Mexico. Avian Dis. 4:310.

Fritzsche, K.: 1961 Aktuelle Fragen der Ornithose Jahreskongress 1960 fur Arztliche Portbilding. 16 75.

——, and Gerrets, E.' 1959. Geflugel Krankheiten. Paul Parey, Berlin, 378 pp.

——, Lippelt, H., and Weyer, F.: 1956 Beitrage zur Epidemiologie. Diagnose und Therapie der Ornithose bei Tauben, Med. Monach tierarzt Wochenschr. 69.61.

Furness, G., and Csonka, G. W.: 1963. A study by fluorescent microscopy of the replication of lymphogranuloma-venereum virus in HeLa cell mono layers. Jour. Gen. Microbiol. 31:161.

Fürst, W., Kovac, W., and Moritsch. H.: 1957 Eaten ais Virus reservoir fur Ornithosererkrankungen des Menschen Wien. klin. Wochenschr. 69:223.

Gale, C.: 1959. The susceptibility of a turkey ornithosis virus of low virulence to antibiotics. Avian Dis. 3.170

——: 1960. Characteristics of an ornithosis virus strain of low virulence in turkeys. Am. Jour. Vet. Res. 21:488.

——: 1961. Ornithosis in turkeys. Proc. 64th Ann. Meet , U.S Livestock Sanit. Assn., Charleston, 1960, p. 223.

——, Pomeroy, B. S., and Sangex, V. L.' 1959 Characterization in mice of a turkey ornithosis virus of low virulence. Jour. Infect Dis. 104:295

——, Sanger V. L., and Pomeroy, B. S.: 1960 The gross and microscopic pathology of an ornithosis virus of low virulence for turkeys. Am. Jour. Vet. Res 21:549.

Gaylord, W. H., Jr.: 1954. Intracellular forms of meningopneumonitis virus. Jour. Exper. Med. 100:575.

Gerloff, R. K., and Lackman, D. B: 1954 Observations regarding the presence of psittacosis and related viruses in the Northwestern States. Am. Jour. Pub. Health 44:523.

Girardi, A. J., Allen, E. G., and Sigel, M. M.: 1952. Studies on the psittacosis-lymphogranuloma group. II. A noninfectious phase in virus development following adsorption to host tissue. Jour. Exper. Med. 96:233.

Giroud, P., and Jadin, J.: 1954. Importance des microagglutinations pour le diagnostic sérologique des infections provoquées par des éléments virulents à la limite des rickettsies. Compt. Rend. Soc. Biol. 148:1157.

Glage, F.: 1930. Über Psittakose. Deutsche tierärztl. Wochenschr. 38.693.

Gneuss, W., and Kottzsch, K. D.: 1961. Über eine Epidemie von Ornithose mit Länfiren Residiven. Deutsch. Gesundh. 16:964.

Gogolak, F. M.: 1953. Purification of murine pneumonitis virus from mouse lung. Jour. Infect. Dis. 92:248.

———: 1953. The histopathology of murine pneumonitis infection and the growth of the viruses in the mouse lung. Jour. Infect. Dis. 92:254.

———: 1954. The mouse erythrocyte hemagglutinin of feline pneumonitis virus. Jour. Infect. Dis. 93:220.

———, and Ross, M. R.: 1955. The properties and chemical nature of the psittacosis virus hemagglutinin. Virology 1:474.

———, and Weiss, E.: 1950. The effect of antibiotics on agents of the psittacosis-lymphogranuloma group. II. The effect of aureomycin. Jour. Infect. Dis. 87:254.

Gönnert, R.: 1942. Ueber einige Eigenschaften des Broncho pneumonievirus der Maus. Zentralbl. f. Bakt., I. Orig. 148:331.

Gordon, F. B.: 1962. The biology of the trachoma agent. Ann. N.Y. Acad. Sci. 98:1.

———, Andrew, V. W., and Wagner, J. C.: 1957. Development of resistance to penicillin and to chlortetracycline in psittacosis virus. Virology 4:156.

———, Bloom, H. H., and Mamay, H. K.: 1960. Studies with drug-resistant strains of psittacosis virus. 1. Comparison of four strains used in mixed cultures. Virology 11:474.

———, Mamay, H. K., and Triemer, R. W.: 1960. Studies with drug-resistant strains of psittacosis virus. II. Derivation of strains with dual drug resistance from mixed culture of singly resistant strains. Virology 11:486.

Gordon, I.: 1958. The diagnosis of human infection. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 139-49.

Graber, R. E.: 1957. Ornithosis-psittacosis in Wisconsin. A preliminary report of a human outbreak transmitted from turkeys. Wisconsin Med. Jour. 56:341.

———: 1959. Epidemiology of turkey-borne ornithosis in Wisconsin. Paper read at the American Public Health Association meeting in Atlantic City, 1959. Mimeographed, 10 pp.

———, and Pomeroy, B. S.: 1958. Ornithosis (psittacosis) an epidemiological study of a Wisconsin human outbreak transmitted from turkeys. Am. Jour. Pub. Health 48:1469.

Grahnéis, H., and Horn, K.: 1961. Hygienisch-epidemiologische Massnahmen als Prophylaxie bei Ornithoseerkrankungen in Geflügelzucht-, Mast- und Sohlachtbetrieben. Deutsch. Gesundh. 16:1602.

Grantova, H., and Milek, E.: 1962. Ornithoseepidemie in einem Geflügelschlachthof. Zeitschr. f. ärztl. Fortbildung 56.897.

———, Vojtech, K., and Milek, E.: 1963. (Pulmonary form of ornithosis). Čas. lék. česk. 102:585

Grattan, D. A. P.: 1963. Ornithosis in turkeys in Britain. Vet. Rec. 75:409.

Greenland, R. M.: 1961. A nonlethal mutant of penicillin-resistant feline pneumonitis virus. Jour. Infect. Dis. 108:287.

Grubb, R.: 1955. Fall av ornithos. Sven. lak. tidn. 52:26.

Güthert, H.: 1938. Die alveolarzellige Pneumonie bei Psittakose. Virchows Arch. f. path. Anat. 302.707.

Haagen, E., and Krickeberg, S.: 1937. Zum Psittakoseproblem. Betrachtungen auf Grund von Beobachtungen und Untersuchungen im Jahre 1935/1936. Veröffentl. a. d. Geb. d. Volkagesundh. 48.381.

———, and Mauer, G.: 1938. Ueber eine auf den Menschen übertragbare Viruskrankheit bei Sturmvögeln und ihre Beziehung zur Psittakose. Zentralbl. f. Bakt., I. Orig. 143:81.

Hamel, C.: 1932. Quelques cas récents de psittacose en Allemagne. Bull. Office internat. d'hyg. pub., 24.966.

Hamre, D. M., Rake, H., and Rake, G.: 1947. Morphological and other characteristics of the agent of feline pneumonitis grown in the allantoic cavity of the chick embryo. Jour. Exper. Med. 86:1.

Hansen, P. F., and Sørensen, L. B.: 1955. Interhuman transmission of ornithosis. Danish Med. Bul. 2:51.

Haussmann, H. G., Siegert, R., Ungar, W., and Ruof, H.: 1956. Beiträge zur Psittakose- (Ornithose-) Infektion des Menschen. Epidemiologische seuchenhygienische und sero-diagnostische Erfahrungen bei einigen Gruppen-Epidemien in Hessen. Arch. f. Hyg. u. Bakt. 140-52.

Hemmes, F., and Golub, O. J.: 1948. Observations on the growth of psittacosis virus in chorioallantoic membranes by electron microscope. Jour. Bact. 56:509.

Hellerstrom, S., and Wassén, E.: 1930. Meningo enzephalitische Veränderungen bei Affen nach intracerebraler Impfung mit Lymphogranuloma inguinale. Compt. Rend. Internat. Dermat. Syph., pp. 1147–51.

Henneberg, G.: 1960. Untersuchungen uber Psittakose. Zentralbl. f. Bakt., I. Orig. 179:29.

Higashi, N., Tamura, A., and Iwanaga, M.: 1962. Developmental cycle and reproductive mechanism of the meningopneumonitis virus in strain L cells. Ann. N.Y. Acad. Sci. 98:100.

Hilleman, M. R.: 1945. Immunological studies on the psittacosis-lymphogranuloma group of viral agents. Jour. Infect. Dis. 76:96.

———. 1955. Serologic procedure for detecting psittacosis infection in birds. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 74–79.

———. Haig, D. A., and Helmold, R. J.: 1951. The indirect complement fixation, hemagglutination and conglutinating complement absorption tests for viruses of the psittacosis-lymphogranuloma venereum group. Jour. Immunol. 66:115.

Hines, M. P., Page, P. M., Hirschberg, N., and Maddry, L. G.: 1957. Ornithosis and leptospirosis survey of a chicken and turkey processing plant and textile mill in North Carolina. Vet. Med. 52:331.

Hornus, G. J. P.: 1940. Psittacose pulmonaire expérimentale de la souris blanche. Ann. Inst. Pasteur 64:97.

Horsfall, F. L., Jr.: 1939. Neutralization of epidemic influenza virus. Linear relationship between the quantity of serum and the quantity of virus neutralized. Jour. Exper. Med. 70:209.

Hudson, G. A., Bivin, J. A., Beaudette, F. R., and Tudor, D. C.: 1955. Use of the chicken embryo technique for diagnosis of psittacosis in avian hosts, with serological notes. Jour. Am. Vet Med Assn 126:111.

Hughes, D. L.: 1947. Ornithosis (psittacosis) in a pigeon flock. Jour. Comp. Path. and Therap. 57:57.

Hurst, E. W., Landquist, J. K., Melvin, P., Peters, J. M., Senior, N., Silk, J. A., and Stacey, G. J.: 1953. The therapy of experimental psittacosis and lymphogranuloma venereum (inguinale). II. The activity of quinoxaline-1:4-dioxide and substituted and related compounds, with a note on the morphological changes induced in lymphogranuloma virus by these compounds and by antibiotics. Brit Jour. Pharmacol. 8:297.

Il'inskii, IU. A., and Dareva, N. P.: 1963. (Clinical and epidemiological data to the problem of the mechanics of ornithosis infection). Vop. Virusol. 8.519.

Illner, F.: 1961 Über das Vorkommen des Ornithosevirus beim Haussperling (Passer domesticus). Monatsh f Veterinärmed. 16 933

———. 1962a Zur Frage der Übertragung des Ornithosevirus durch das El. Monatsh. f. Veterinärmed 17.116.

———. 1962b Ein Beitrag Zur Enten Ornithose und ihrer Epizootiologie. Monatsh. f. Veterinärmed. 17:141

Inaba, Y., Omori, T, Ishii, S, and Matumoto, M.: 1957 Miyagawanella psittacosis-lymphogranuloma group of viruses. 3. Hemagglutination of psittacosis virus Jap. Jour. Exper. Med 27:423

———. Omori, T., Morimoto, T, Kurogi, H, Kono, V., Ishii, S, and Matumoto, M.: 1958. Miyagawanella psittacosis lymphogranuloma group of viruses. 7 Psittacosis virus in Japanese pigeons. Jap. Jour Exper. Med. 28:225.

Irons, J. V, Denley, M. L., and Sullivan, T. D.: 1955 Psittacosis in turkeys and fowls as a source of human infection. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 44–65.

———. Sullivan, T. D., and Rowen, J.: 1951. Outbreak of psittacosis (ornithosis) from working with turkeys or chickens. Ann. Jour. Pub Health 41 931.

Jacobi, H. R.: 1957. Effect of ornithosis on experimental fowl malaria. Proc Soc. Exper. Biol and Med. 95:372.

Jansen, J.: 1955 L'ornitosi nei piccioni e sue ripercussioni nel campo umano. Zooprofilassi 10 495.

———. 1955. Ornithosis in pigeons. VIIme Cong. Internat. de Path. Comp., Lausanne 1:13.

———. 1959 Ornithosis bij duiven. Tijdschr. Diergeneesk. 81 643

Jansson, E.: 1960. Ornithosis in Helsinki and some other localities in Finland. A serological and clinical study. Ann. Med. Exper. et Biol. Fenniae 38, 110 pp

Jawetz, E., and Thygeson, P.: 1964. TRIC viruses: Agents of trachoma and inclusion conjunctivitis Ergeb d Mikrobiol 38 55

Jenkin. H. M.: 1960 Preparation and properties of cell walls of the agent of meningopneumonitis. Jour Bact. 80.639.

———. Ross, M R., and Moulder, J. W.: 1961. Species specific antigens from the cell walls of the agents of meningopneumonitis and feline pneumonitis. Jour. Immunol. 86:123.

Johnson, K. M., and Morgan, H. R.: 1956. Latent viral infection of cells in tissue culture. 11. Relationship of cell nutrition to initiation of growth of psittacosis virus. Jour. Exper. Med 103.765.

Jørgensen, J. H., Søndergaard, E., and Volkert, M.: 1963. Studies on the chemical nature of ornithosis complement fixing antigen. Acta Pathol et Microbiol. Scandinav. 57:111.

Kalra, S. L.: 1958. Ornithosis in man and in indigenous fowls in India. Indian Jour. Med. Sci. 12:162.

Karr, H. V.: 1943. Study of a latent pneumotropic virus of mice. Jour. Infect. Dis. 72:108.

Karrer, H., Eddie, B., and Schmid, R.: 1950. Barnyard fowl as a source of human ornithosis Case report. California Med. 73:55.

———, Meyer, K. F., and Eddie, B.: 1950a The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks I. Principles and technique of the test. Jour. Infect. Dis. 87:13.

———, Meyer, K. F., and Eddie, B.: 1950b. The complement fixation inhibition test and its application to the diagnosis of ornithosis in chickens and in ducks. II. Confirmation of the specificity and epidemiological application of the test. Jour. Infect. Dis. 87:24.

Katz, E.: 1956. The activity of tetracycline on feline pneumonitis virus infection of chick embryos. Jour. Infect. Dis. 98:177.

Kemmerer, G., Haussmann, H. G., Schoop, G., and Kauker, E.: 1956. Zur Klinik und Epidemiologie der durch Tauben übertragenen menschlichen Ornithose Deutsche med Wochenschr. 81:930.

Keymer, I. F.: 1959. Psittacosis. Vet. Rec. 71:354.

Kiehlstein, P.: 1961. Pathogenese, Epidemiologie und Bekämpfung der Ornithose. Berl. Münch. tierärztl. Wochenschr. 74:440.

Kissling, R. E., Schaeffer, M., Fletcher, O. K, Stamm, D. D., Bucca, M. A., and Sigel, M. M.: 1956. Diagnosis of psittacosis in parakeets. Pub. Health Rep. 71:719.

Klitek: 1955. Ornithosis in Norddeutschland. Desinfek. u. Gesundh. 47:65.

Komarov, A., and Goldsmit, L.: 1952. The presence of the causative agent of ornithosis in Israel. Harefuah 42:6.

Koppel, Z., and Polony, R.: 1958 (Remarks on the etiology of mass dying of water poultry coincidence of ornithosis and salmonellosis in poultry farms). Vet. Čas 7:205.

Korns, R. F.: 1955. Psittacosis in ducks and persons exposed to ducks. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 80–89.

Kortev, A I., and Fedorova, A. S.: 1963. (Ornithosis in the Sverdlov oblast), Sovet. Med. 26:124.

Kovac, W.: 1961. Zur Entwicklung und Spezifität der Psittakose im Tierexperiment. I. Mitteilung. Zentralbl. f. Bakt., I., Orig. 181:175.

Kožulinak, Z.: 1960 K otázce výskytu ornithosy ve východních Čechách (Psittacosis in Eastern Bohemia). Sborn. čes. Akad. zemědělsk. Věd, vet. Med. 5:789.

Krivinka: 1959. Die Ornithose der Enten. Proc. 16th Internat. Vet. Cong., Madrid 2:565.

Krumwiede, C., McCrath, M., and Oldenbusch, C.: 1930. The etiology of the disease psittacosis Science 71.262.

Kubášek, M., and Strauss, J.: 1956. Epidemic and epizootic appearance of ornithosis in one district. English abstract 2 on Ornithosis presented at the Congress on Anthropozoonotes held in Prague on May 22, 1956, 1 p.

———, and Strauss, J.: 1956. Epidemický A Epizootický Výskyt Ornithosy V Jednom Okrese. Přednešeno na 10 sjezdu čs. mikrobiologů a epidemiologů čs lékařské spol. J. Ev. Purkyně a Ornithopozoonosách. v Praze 22. 5.

Kuyumgieff, I.: 1957. The spread of ornithosis in Bulgaria Acta Virol. 1:57.

Kukowka, A.: 1961a. Aktualitat der Ornithosen. Zeitschr. f. d. ges. inn. Med. u. Grenzgeb. 16:257.

———: 1961b. Zur Prophylaxe von Ornithosen Deutsche Gesundh. 16:441.

———: 1961c. Pneumonien bei Geflügel-Ornithose-erkrankungen. Medizinische 10:240.

———, and Stephan, H.: 1961. Über 23 im Jahre 1959 in der Infektionsabteilung des Krankenhauses Greiz (Thüringen) beobachtete Fälle von Ornithose. Zeitschr. f. ärztl. Fortbildung 55:584.

———, Stephan, H., and Krebs, W.: 1960. Entenfarm als Ausgangspunkt von Ornithoseerkrankungen bei Menschen. Deutsche Gesundh. 15:2477.

Labzoffsky, N. A.: 1946. Rapid agglutination test as possible aid in laboratory diagnosis of ornithosis. Jour. Infect. Dis. 79.96.

———: 1947. Ornithosis among "wild" pigeons in Ontario. Canad. Jour. Pub. Health 38-187.

Lazarus, A. S., and Meyer, K. F.: 1939. The virus of psittacosis. III. Serological investigations. Jour. Bact. 38:171.

Leachman, R. D., and Yow, E. M.: 1958. The epidemiology of psittacosis and report of a turkey-borne outbreak. Ann. Int. Med. 102:557.

Lehnert, C.: 1962. Zur Frage der Übertragung des Ornithose virus über das Brutei bei Enten. Berl. Münch. tierärztl. Wochenschr. 75:151.

———, and Hille, G.: 1960. Serologische Ornithose-Untersuch ungen in Zucht-und Mastentenbestanden. Berl. Münch. tierärztl. Wochenschr. 73:441.

Lépine, P., and Sautter, V.: 1951. Sur l'infection des pigeons parisiens par le virus de l'ornithose. Bull. Acad. Nat. Méd. 135:332.

Levi, W. M.: 1960. Domesticated pigeons do not carry or spread diseases to human beings—facts exonerate pigeons of false charges. Am. Pigeon Jour. January 1961, 4 pp.

Levinson, D. C., Gibbs, J., and Beardwood, J. T., Jr.: 1944. Ornithosis as a cause of sporadic atypical pneumonia. Jour. Am. Med. Assn. 126-1079.

Levinthal, W.: 1930. Die Ätiologie der Psittakosis. Klin. Wochenschr. 9.654.

Lillie, R. D.: 1933. I. The pathology of psittacosis in man. II. The pathology of psittacosis in animals and the distribution of Rickettsia psittaci in the tissues of man and animals. Nat. Inst. Health Bul. 161, Washington, D.C., Pp. 1–66.

Lippi, M., Frugoni, G., and Benedetto, A.: 1960. Contributo sierolmmunologico alla conoscenza delle principali virosi. Ricerca nei soggetti sani degli anticorpi devianti il complemento verso il virus dell'ornitosi. Arch. Ital. Sci. Med. Trop. e Parassit. 41:563.

Litwin, J.: 1957. A simple method for cultivation of viruses and rickettsiae in the chorioallantoic ectoderm of the chick embryo by inoculation via the air sac. Jour. Infect. Dis. 101:100.

——: 1959 The growth cycle of the psittacosis group of microorganisms. Jour. Infect. Dis. 105.129.

——: 1962 Growth of the agent of trachoma in the embryonated egg. Ann. N.Y. Acad. Sci. 98:145.

——, Officer, J. E., Brown, A., and Moulder, J. W.: 1961. A comparative study of the growth cycles of different members of the psittacosis group in different host cells. Jour. Infect. Dis. 109 251.

Loosli, C. G., and Ritter, M. H.: 1948. The pathogenesis and histopathology of air-borne pneumonitis virus infection in mice. The effect of penicillin G upon the developing lesion. Jour. Clin. Investigation 27:549 (Abstract).

McCulloh, A.: 1951. An epidemic of psittacosis in poultry workers. Clinical evaluation and treatment. Texas State Jour Med. 51:817.

McEwen, A. D., Dow, J. B., and Anderson, R. D.: 1955. Enzootic abortion in ewes. An adjuvant vaccine prepared from egg. Vet. Rec. 67:594.

——, and Foggie, A.: 1956. Enzootic abortion in ewes. Prolonged immunity following the injection of adjuvant vaccine. Vet. Rec. 68.686.

——, Littlejohn, A., and Foggie, A.: 1951. Enzootic abortion of ewes: some aspects of infection and resistance. Vet. Rec. 63:489.

McGavran, M. H., Beard, C. W., Berendt, R. F., and Nakamura, R. M.: 1962. The pathogenesis of psittacosis Serial studies on rhesus monkeys exposed to a small particle aerosol of the Borg strain. Am. Jour. Path. 40.653.

Mach, R. S., Fallet, G. H., and Sautter, V.: 1950. Un cas d'ornithose. Bul. et mem. Soc. hôp. Paris 66.561

Mack, W. N.: 1955 The isolation of ornithosis virus (psittacosis) from a turkey in Michigan. Michigan State Univ. Vet. East Lansing 16.12.

Maerae, A. D 1951 Psittacosis: some recent developments. Jour. Roy. Sanit. Inst. 71:121.

Mandel, A., and Jordan, W. S., Jr.: 1952. Ornithosis (psittacosis) in chickens and poultry workers. Am. Jour. Hyg 55:250

Manire, G P, and Galasso, G J: 1959. Persistent infection of HeLa cells with meningopneumonitis virus Jour Immunol 83.529

——, and Meyer, K. F.: 1950a. The toxins of the psittacosis-lymphogranuloma group of agents I. The toxicity of various members of the psittacosis-lymphogranuloma venereum group. Jour Infect. Dis 86.226

——, and Meyer, K. F.: 1950b The toxins of the psittacosis-lymphogranuloma group of agents. II Effect of suramycin and penicillin upon the toxins of psittacosis viruses. Jour. Infect. Dis 86:253.

——, and Meyer, K. F.: 1950c The toxins of the psittacosis-lymphogranuloma group of agents III Differentiation of strains by the toxin neutralization test. Jour. Infect. Dis. 86.241.

——, and Smith, K O.: 1959 Quantitative studies of meningopneumonitis virus. Jour. Bact. 78:523.

Marinescu, G., Sarateanu, D., and Hung. T.: 1960. Comparative studies on histological changes in white mice infected with various autochthonous strains of the ornithosis virus. Stud. Cercet. Inframicrobiol. 11:219.

Mason, D M.: 1959. A capillary tube agglutination test for detecting antibodies against ornithosis in turkey serum. Jour. Immunol 83 661.

Matthiessen, M.: 1956. Two hundred and forty-four patients with positive ornithosis complement fixation tests. Age, sex, and seasonal distribution. Danish Med. Bul. 3:248.

Matumoto, M., Goto, T., Nakamura, H., Matsushima, S., Nano, H., Shioda, H., and Omori, T.: 1960. Psittacosis in Japan. Analysis of patients with primary atypical pneumonia, acute bronchitis and other diseases by means of complement fixation and hemagglutination inhibition tests Japanese Jour. Exper. Med. 30:327.

——, Omori, T., Morimoto, T., Harada, K., Inaba, Y., and Ishii, S.: 1955. Studies on the disease of cattle caused by bovine PL virus, a psittacosis-lymphogranuloma group virus. Japanese Jour. Exper. Med. 25:229.

Meenan, P. N., Clarke, M., and Brenn, D. S.: 1950. Occurrence of psittacosis (ornithosis) in Irish pigeons. Jour. Med. Assn. Eire 26 70

Melzer, H.: 1959. Zur Epidemiologie und Klinik der Ornithose. Deutsche med. Wochenschr. 84:664.

Meyer, K. F.: 1935. Psittacosis. Proc. 12th Internat. Vet. Cong. 3:182.

————: 1941. Pigeons and barn yard fowls as possible sources of human psittacosis or ornithosis. Schweiz. med. Wochenschr. 71:1377.

————: 1942. The ecology of psittacosis and ornithosis. Medicine 21:175.

————: 1952. Reservoirs of the psittacosis agent. Acta Tropica 9:204.

————: 1953a. Psittacosis group. Ann. N.Y. Acad. Sci. 56:545.

————: 1955b. Distribution and identification of psittacosis viral agents in the animal kingdom. Proc. 15th Internat. Vet. Cong. Stockholm 1:338.

————: 1954. Early diagnosis of infections by the psittacosis-lymphogranuloma venereum group. In: The Dynamics of Virus and Rickettsial Infections, ed. by F. W. Hartman, F. L. Horsfall, Jr., and J. G. Kidd. The Blakiston Company, New York, pp. 295–323.

————: 1955. Problems in the control of psittacosis and ornithosis. Proc. Book, Am. Vet. Med. Assn., 92nd Meet., pp. 412–19.

————: 1958. Ornithosis: A public health problem. 62nd Proc. U.S. Livestock Sanit. Assn., pp. 230–43.

————: 1959a. Some general remarks and new observations on psittacosis and ornithosis. Bul. World Health Organ. 20:101.

————: 1959b. Ornithosis. In: Diseases of Poultry, ed. by H. E. Biester and L. H. Schwarte. 4th ed. Iowa State University Press, Ames, Iowa, pp. 526–27.

————: 1962. Antimicrobial therapy and prophylactic immunization in the control of psittacosis or bedsonia infection in show birds. Schweiz. med. Wochenschr. 92:1682.

————: 1964. Evolution of the problems of the occupational diseases acquired from animals. In: Occupational Diseases Acquired From Animals, Continued Education Series No. 124, Ann Arbor, Michigan, University of Michigan, School of Public Health, pp. 4–85.

————, and Eddie, B.: 1933. Latent psittacosis infections in shell parakeets. Proc. Soc. Exper. Biol. and Med. 30:484.

————, and Eddie, B.: 1939a. The value of the complement fixation test in the diagnosis of psittacosis. Jour. Infect. Dis. 65:225.

————, and Eddie, B.: 1939b. Psittacosis in importations of psittacine birds from the South American and Australian continents. Jour. Infect. Dis. 65:234.

————, and Eddie, B.: 1942. Spontaneous ornithosis (psittacosis) in chickens the cause of a human infection. Proc. Soc. Exper. Biol. and Med. 49:522.

————, and Eddie, B.: 1951a. A review of psittacosis for the years 1948 to 1950. Bul. Hyg. 26:1.

————, and Eddie, B.: 1951b. Human carrier of the psittacosis virus. Jour. Infect. Dis. 88:109.

————, and Eddie, B.: 1952. Reservoirs of the psittacosis agent. Acta Tropica 9:204.

————, and Eddie, B.: 1955 Characteristics of a psittacosis viral agent isolated from a turkey. Proc. Soc. Exper. Biol. and Med. 83:99.

————, and Eddie B.: 1954a. Unpublished official reports.

————, and Eddie, B.: 1954b. Field trials of antibiotic treatment of psittacosis and ornithosis. All-Pets Magazine 25(11):74.

————, and Eddie, B.: 1955a. Chemotherapy of natural psittacosis and ornithosis. Field trial of tetracycline, chlortetracycline, and oxytetracycline. Antibiotics and Chemotherapy 5:289

————, and Eddie, B.: 1956a. Unpublished data. (Isolation of the bedsonia from pheasants.)

————, and Eddie, B.: 1956b. Unpublished data. (Ornithosis due to a bedsonia of low virulence)

————, and Eddie, B.: 1956c. The influence of tetracycline compounds on the development of antibodies in psittacosis. Am. Rev. Tuberc. 74:566.

————, and Eddie, B.: 1958a. Unpublished data. (Laboratory infections)

————, and Eddie, B.: 1958b. Unpublished data. (Drug sensitive turkey isolate.)

————, and Eddie, B.: 1958c. Ecology of avian psittacosis, particularly in parakeets. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 52–79.

————, and Eddie, B.: 1959. Unpublished data. (Ornithosis in turkeys in Michigan)

————, and Eddie, B.: 1961. Human carrier of the psittacosis virus. Jour. Infect. Dis. 88:109.

————, and Eddie, B.: 1962a. Immunity against some bedsonia in man resulting from infection and in animals from infection and vaccination. Ann. N.Y. Acad. Sci. 98:288.

————, and Eddie, B.: 1962b. Unpublished data. (Isolate of low virulence from Oregon turkeys.)

————, Eddie, B., Richardson, J. H., Shipkowitz, N. L., and Muir, R. J.: 1958. Chemotherapy in the control of psittacosis in parakeets. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 165–96.

————, Eddie, B., and Yanamura, H. Y.: 1939. Complement-fixation test with tissue-culture antigens as aid in recognizing latent avian psittacosis (ornithosis). Proc. Soc. Exper. Biol. and Med. 41:175.

————, Eddie, B., and Yanamura, H. Y.: 1942a. Ornithosis (psittacosis) in pigeons and its relation to human pneumonitis. Proc. Soc. Exper. Biol. and Med. 49:609.

Meyer, K. F., Eddie, B., and Yanamura, H. Y.: 1929. Active immunization to *Microbacterium psittacosis* in parakeets and lovebirds. Jour. Immunol. 16:211.

Meyer, P. G., and Geensens, R. J.: 1957. Klinischer Beitrag zum Problem der Ornithose (Psittakose). Helvet. Med. Acta 24:127.

Michael, K. P.: 1956a. The microbiology and epidemiology of psittacosis-ornithosis. Part I. Acta Microbiol. Hellenica 1:229.

———: 1956b. Psittacosis-ornithosis from the etiological and epidemiological point of view. Part II. Acta Microbiol. Hellenica 1:505.

———: 1957. Studies on ornithosis in Greece. Acta Microbiol. Hellenica 2:14.

Miles, J. A. R.: 1951. Observations on complement fixation and complement fixation inhibition using certain avian era. Australian Jour. Exper. Biol. and Med. Sci. 29:413.

———: 1955a. Enzootic ornithosis (psittacosis) in New Zealand. New Zealand Med. Jour. 58:506.

———: 1959b. Ornithosis research in Australia. Biogeog. and Ecol. in Australia. Monograph. Biol. 8:412.

———, and Shrivastav, J. B.: 1951. Ornithosis in certain sea-birds. Jour. Anim. Ecol. 20:195.

Mitscherlick, E.: 1955. Beiträge zum Virusabort des Schafes. 1 Mitteilung: Die Ätiologie des Virusabortes des Schafes. Vet. Med. Nachrichten 1:129.

Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., and Okanishi, J.: 1935. Studies on virus of lymphogranuloma inguinale Nicolas, Favre and Durand. II, III, IV, V. Jap. Jour. Exper. Med. 13:331.

Mohr, W.: 1941. Untersuchungen und Beobachtungen zur Verbreitung und Klinik der Ornithose (Psittakose) in Deutschland. Zeitschr. f. d. ges. inn. Med. u. Grenzgeb. 9:1005.

Molitke, O.: 1932. Psittacosis. 4 cases. Ugesk. f. lager 94:78.

Monreal, G.: 1958. Untersuchungen über den direkten und indirekten Virusnachweis bei der Ornithose der Tauben. Zentralbl. f. Veterinärmed. 5:273.

———: 1959. Untersuchungen über Veruchen durch Hausund Schlachtgeflügel aus der Sicht der Veterinärmedizin. Der Landarzt 35:176.

———: 1960. Systematische Untersuchungen von Wellensittichbeständen und Pathogenitätsprüfung verschiedener Psittakosestämme mit Hilfe der Maus versuchen. Mb. Tierh. K. 12:101.

———: 1963. Künftige Aspekte bei der Bekämpfung der Ornithose (Psittakose). Zentralbl. f. Veterinärmed 10:291.

Monsur, K. A., and Barwell, C. F.: 1951. Observations on the antigenic relationship between the virus of enzootic abortion in ewes and viruses of the psittacosis lymphogranuloma group. Brit. Jour. Exper. Path. 32:414.

Moore, R. W., and Watkins, J. R.: 1960. The comparative effects of chlortetracycline and oxytetracycline in the treatment of turkeys with ornithosis. Jour. Am. Vet. Med. Assn. 136:563.

Morgan, H. R.: 1951. Factors related to the growth of psittacosis virus (strain 6BC). IV. Certain amino acids vitamins and other substances. Jour. Exper. Med. 29:451.

———, and Bader, J. P.: 1956. Latent viral infection of cells in tissue culture. I. Studies on latent infection of chick embryo tissues with psittacosis virus. Jour. Exper. Med. 103:37.

———, and Bader, J. P.: 1957. Latent viral infection of cells in tissue culture. IV. Latent infection of L cells with psittacosis virus. Jour. Exper. Med. 106:39.

———, and Wiseman, R. W.: 1946. Growth of the psittacosis virus in roller tube tissue culture. Use in vaccine. Jour. Infect. Dis. 79:131.

Morimoto, T., Omori, T., Ishii, S., and Matumoto, M.: 1958. Miyagawanella: psittacosis-lymphogranuloma group of viruses. 6. Detection of indirect complement fixing antibodia against psittacosis in chicken serum. Jap. Jour. Exper. Med. 28:129.

Morisch, H. and Kosac, W.: 1956. Virologischer und histologischer Organbefund eines Wellensittichs bei Psittacosis. Schweiz. Zeitschr. f. allge. Path. u. Bakt. 19:182.

Morrissey, R. A., and Meyer, K. F.: 1951. Unpublished data. (Isolation of the virus from a pheasant).

Moulder, J. W.: 1955. Biochemical aspects of the growth of feline pneumonitis virus in the chick embryo yolk sac. Bact. Rev. 18:170.

———: 1962a. The Biochemistry of Intracellular Parasitism. University of Chicago Press, Chicago, Illinois, 172 pp.

———: 1962b. Some basic properties of the psittacosis lymphogranuloma vertreum group of agents. Structure and chemical composition of isolated particles. Ann. N.Y. Acad. Sci. 98:92.

———: 1964. The psittacosis group as bacteria. Ciba Lectures in Microbial Biochemistry. John Wiley & Sons, Inc., New York, 95 pp.

———, Colón, J. I., Ruda, J., and Zebovitz, M. M.: 1956. The effect of penicillin on multiplying and non-multiplying population of sensitive and resistant strains of feline pneumonitis virus. Jour. Infect. Dis. 98:229.

McCormack, D. B. S., Gogolak, F. M., Zebovitz, M. M., and Itatani, M. K.: 1955. Production and properties of a penicillin-resistant strain of feline pneumonitis virus. Jour. Infect. Dis. 96:57.

———, Ruda, J., Colón, J. I., and Greenland, R. M.: 1958. The effect of passage with chloramphenicol upon the behavior of penicillin-resistant feline pneumonitis virus during subsequent passage with penicillin. Jour. Infect. Dis. 102:186.

————, and Weiss, E.: 1951. Purification and properties of the agent of feline pneumonitis. Jour. Infect. Dis. 88.56.

Mumme, C.: 1955. Zur Epidemiologie und Klinik der Ornithose. Verh. d. Deutsch. Gesellsch. f. inn. Med. 44:244.

Murray, D. S.: 1960. Ornithosis in railway guards. Lancet 1:101.

Mykytowycz, R., Dane, D. A., and Beech, M.: 1955. Ornithosis in the petrel, *Puffinus tenuirostris* (Temminck). Australian Jour. Exper. Biol. and Med. Sci. 33:629.

Neal, J. E., and Davis, D. E.: 1958. A comparison of the indirect complement-fixation test, the direct complement-fixation test, and the macroscopic agglutination test for ornithosis antibodies in turkey serums. Am. Jour. Vet. Res. 19:200.

Neustroev, V. D., Khanduev, T. T., and Mihutin, V. N.: 1958. The use of fluorescence microscopy for demonstrating ornithosis virus in the organs of infected animals. Prob. Virol. 3:158.

————, Khanduev, Z. Z., and Mihutin, V. N.: 1959. The counting of the elementary bodies of ornithosis virus by fluorescence microscopy. Prob. Virol. 4:106.

Nichols, R. L., and McComb, D. E.: 1962. Immunofluorescent studies with trachoma and related antigens. Jour. Immunol. 89:545.

Nigg, C., and Eaton, M. D.: 1944. Isolation from normal mice of a pneumotropic virus which forms elementary bodies. Jour. Exper. Med. 79:497.

Officer, J. E., and Brown, A.: 1960. Growth of psittacosis virus in tissue culture. Jour. Infect. Dis. 107:283.

Olivo, R., and Badiali, C.: 1956. La diffusione dell' ornitosi tra i piccioni in Provincia di Bologna. Ricerche sierologiche. G. Mal. Infett. Parassit. 8.145.

Orfila, J.: 1962. Étude de l'action cytopathogène du virus de la lymphogranulomatose vénérienne en culture de tissus. Ann. Inst. Pasteur 102:249.

Ormsbee, R. A., and Weiss, E.: 1963. Trachoma agent: glucose utilization by purified suspensions. Science 142:1077.

Ortel, S.: 1960. Serologische und epidemiologische Untersuchungen während einer Ornithose-Epidemie bei Angestellten eines Geflügel-Schlachthofes. Zentralbl. f. Bakt., I, Orig 180:441.

————: 1961. Aktuelles über Ornithose-Psittakose. German Med. Jour. 12:615.

————: 1963. Zur Epidemiologie der Ornithose. Münch. Med. Wochenschr. 105:1105.

————: 1964. Die Ornithose-Situation in der DDR auf Grund epidemiologischer und serologischer Untersuchungen. (Mit 5 Abbildungen.) Arch. Exp. Vet. Med. 18:89.

Osgood, S. B., Holmes, M. A., Mason, D. M., Caplan, G., Meyer, K. F., and Eddie, B.: 1958. Ornithosis in Oregon, 1956. A report of outbreaks in turkeys and man. Mimeographed report by the Oregon State Board of Health, Communicable Disease Center, United States Public Health Service and the George Williams Hooper Foundation, 107 pp.

Otto, H.: 1962. Zur Klinik humaner Ornithosen mit einem Erfahrungsbericht aus zwei Endemien. Zeitschr. f. ärztl. Fortbildung 56:494.

Page, L. A.: 1959a. Experimental ornithosis in turkeys. Avian Dis. 3.51.

————: 1959b. Thermal inactivation studies on a turkey ornithosis virus. Avian Dis. 3.67.

————: 1959c. Measurement of pathogenicity of turkey ornithosis agents for mice. Avian Dis. 3.25.

————: 1960. Ecologic considerations in turkey ornithosis. Am. Jour. Vet. Res. 21:618.

————, and Bankowski, R. A.: 1959. Investigation of a recent ornithosis epizootic in California turkeys. Am. Jour. Vet. Res. 20.941.

————, and Bankowski, R. A.: 1960. Factors affecting the production and detection of ornithosis antibodies in infected turkeys. Am. Jour. Vet. Res. 21.971.

————, Storz, J., and Pangborn, J.: 1961. Electron microscopic survey of thirteen strains of *Miyagawanella*. Avian Dis. 5.121.

Parnas, J.: 1961. Ein Beitrag zur Technik der Komplement-bindungsreaktion (KBR) auf Ornithose. Zeitschr. f. d. ges. Hyg. u. Grenzgeb. 9:76.

————: 1964. Ergebnisse der bisherigen Forschungen über Ornithose. Arch. f. Exp. Veterinärmedizin 18:77.

————, and Simunen, W.: 1961. Untersuchungen über Ornithose. Zentralbl. f. Bakt., I, Orig 183.141.

————, Simunen, W., and Lange: 1960. Ornitoza (choroba ptasia)—nowa choroba pracownicza w hodowli. Med. Pracy. 11:411.

Parodi, A. S., and Silvetti, L. M.: 1946. La psitacosis en los psitácidos silvestres (*Myiopsitta monacha*, Bodde) de la República Argentina. Prensa Méd. Argent. 33.529.

Parry, W. H., and Griffith, A.: 1962. Ornithosis. An epidemiological study in Liverpool. Med. Officer 107:181.

Pate, D. D., Boney, W. A. Jr., and Delaplane, J. P.: 1954. Turkey ornithosis. 1. A report of natural and experimental infections. Jour. Am. Vet. Med. Assn. 125.476.

Peavy, J. E., and Dickerson, M. S.: 1963. Psittacosis—Texas. Morb. and Mort. Weekly Rep. 12 369.

Perkins, H. R., and Allison, A. C.: 1963. Cell-wall constituents of rickettsiae and psittacosis-lymphogranuloma organisms. Jour. Gen. Microbiol. 30.469.

Pilod, M.: 1960. Sur l'importance et le degré de gravité du dangers qui constituent pour la santé publique, les pigeons qui prolifèrent dans la region parisienne, ainsi que les risques eventuels qui comporte pour les personnes l'emploi du gluco chloral (chloralose). Bul. Acad. Nat. Méd. 141:134.

Pinketon, H., and Moragues, V.: 1942. Comparative study of meningopneumonitis virus, psittacosis of pigeon origin, and psittacosis of parrot origin. Jour. Exper. Med. 75:575.

———, and Swank, R. L.: 1940. Recovery of a virus morphologically identical with psittacosis from vitamin-deficient pigeons. Proc. Soc. Exper. Biol. and Med. 45:704.

Pollard, M. Moore, R. W., Swat, T. J., and Tanami, Y.: 1960. Ultraviolet microscopy of psittacosis virus-infected cells treated with antibiotics and with antimetabolites. Antimicrob. Agents Annual 1960 272.

———, and Tanami, Y.: 1962. Cytochemistry of trachoma virus replication in tissue cultures. Am. N.Y. Acad. Sci. 98:50.

Polony, R., Koppel, Z., and Vrtiak, J.: 1960. Epizootologické sledovanie ornitózy na vycrodnom Slovensku. Veterinátsky Casopis 9.476.

Pomeroy, B. S., Brumfield, H., and Bates, H.: 1957. Research program in Minnesota on ornithosis of turkeys. Paper read at the 8th Annual North Central Regional Poultry Disease Conference, University of Minnesota.

Popovici, V., and May, I.: 1960. (Fresh outbreak of psittacosis in ducks) Lucr. Inst. Pat, Igiena Anim, Bucuresti 10:119. Abstract: Vet. Bul. 31, F 199 1961.

———, Stoenescu, V, Sandulescu, St., and Stefanescu, Tr.: 1959. (Isolation of virus strain of P. L.-ornithosis from ducks.) Lucr. Inst. Pat. Igiena Anim., Bucuresti 9.103.

Rake, G., and Jones, H. P.: 1942. Studies on lymphogranuloma venereum. I. Development of the agent in the yolk sac of the chicken embryo. Jour. Exper. Med. 75:323.

———, and Jones, H.P.: 1944. Studies on lymphogranuloma venereum. II. The association of specific toxins with agents of the lymphogranuloma-psittacosis group. Jour. Exper. Med. 79:463.

Rasmussen, R. X.: 1938. Ueber eine durch Sturmvogel übertragbase Lungenerkrankung auf den Färoern. Zentralbl. I. Dakt. (Abt. I) 143:89.

Reeve, P., and Graham, D. M.: 1962. A neutralisation test for trachoma and inclusion blennorrhoea viruses grown in HeLa cell cultures. Jour. Gen. Microbiol. 27:177.

Renthein, H., and Walther, G.: 1961. Zur Klinik und Epidemiologie der Psittakose und Ornithose. Internist (Berlin) 2:514.

Rice, C. E.: 1936. Carbohydrate matrix of epithelial-cell inclusion in trachoma. Am. Jour. Ophth. 19 1.

——— : 1948. Inhibitory effects of certain avian and mammalian antisera in specific complement-fixation systems. Jour. Immunol. 59:365.

——— : 1961. The use of complement fixation tests in the study and diagnosis of viral diseases in man and animals—a review. VII. The psittacosis lymphogranuloma venereum group. Canad. Jour. Comp. Med. 25:74.

Rich, A. R.: 1962. Human psittacosis outbreak—Texas. CDC Vet. Pub. Health Newsletter, pp. 17–18.

———, Pessara, J., and Dickerson, M. S.: 1962. Psittacosis and Hurricane Carla—Texas. Morb. and Mort. Weekly Rep. 11:54.

Rindge, M. E., Jungherr, E. L., and Scruggs, J. H.: 1959. Serologic evidence of occupational psittacosis in poultry-plant workers. New England Jour. Med. 260:1214.

Ringleben, H.: 1960. Verwildert Haustauben in hygienischer Sicht. (The pigeon nuisance in cities) Deutsche u. Gesundh. 829:124.

Rivers, T. M., and Berry, G. P.: 1931. Psittacosis. IV. Experimentally induced infections in monkeys. Jour. Exper. Med. 54:129.

———, Berry, G. P., and Sprunt, D. H.: 1931. Psittacosis I. Experimentally induced infections in parrots. Jour. Exper. Med. 54.91.

———, and Schwentker, F. X.: 1934. Vaccination of monkeys and laboratory workers against psittacosis. Jour. Exper. Med. 60:211.

Roger, F., and Lépine, P.: 1961. Epidémiologie de l'ornithose. Bul. Inst. Pasteur 59:20.

Rosen, B.: 1955. Ornithosis as an occupational hazard. Radiology 65:373.

Ross, M. R., and Gogolak, F. M.: 1957a. The antigenic structure of psittacosis and feline pneumonitis viruses. I. Isolation of complement-fixing antigens with group and species specificity. Virology 3:343

———, and Gogolak, F. M.: 1957b. The antigenic structure of psittacosis and feline pneumonitis viruses. II. Chemical nature of the alkali-soluble antigens. Virology 3.365.

———, and Jenkin, M. M.: 1962. Cell wall antigens from members of the psittacosis group of organisms. Ann. N.Y. Acad. Sci 96.329.

Rudnai, O., Solt, K., and Domok, L.: 1964. Ornithose-Epidemien in Ungarn in den Jahren 1960 bis 1962. Archiv für Exper. Veterinärmed. 18:125.

Rugiero, H.R., Averbach, S., Carlone, M., and Landaburu, J.: 1950. 1) Epidemiología de la psitacosis en la Republica Argentina. Prensa Méd. Argent. 37:2593.

Sacquépée, E., and Ferrabouc, L.: 1930. Sur l'étiologie de la psittacose. Presse Méd. 38:569.

Sarateanu, D.: 1963. Ornitoza. Editura Academici Republicii Populare Romine. Intreprinderea Poligrafica "Informatia," str. Brezoianu nr. 23–25, Bucuresti R.P.R. comanda nr. 2370, 351 pp.

———, Draganescu, N., Portocala, R., and Ionescu, N.: 1958. (Neorickettsial pneumonitis. Isolation and identification of the pathogenic agent) Microbiol., Parazitol., Epidemiol. Bucharest 3.529.

———, Nastac, E., Fuhrer, B., Oprescu, E., and Hung, T.: 1960. (Research on ornithosis virus infection in the Rumanian People's Republic) Stud. Cercet. Inframicrobiol. 11:73.

———, Nastac, E., Fuhrer, B., Sorodoc, G., Surdan, C., and Oprescu, E.: 1961. (The incidence rate of ornithosis antibodies in workers in the zootechnical field.) Stud. Cercet. Inframicrobiol. 12 (suppl.) 365. (English summary.)

Scheidegger, S.: 1953. Experimental viral infections in the embryo and fetus. Preliminary notes on pathologic findings with viruses of psittacosis, ectromelia, and rabies. Am. Jour. Path. 29:185.

———: 1960. Ornithose. Proc. XIIth Internat. Ornithological Cong., Helsinki, 1958, p. 649.

———: 1961. Ornithose. Path. u. Microbiol. 24:259.

Schmidtke, L.: 1957. Psittakose. Entwicklung der epidemiologischen Lage ab 1931. Übersichtsreferat. Zentralbl. f. Bakt., I. Orig. 168:1.

Schmittdiel, E.: 1961. Versuche zur Konservierung des Psittakose-virus durch die Vakuumgefriertrocknung. Zentralbl. Bakt. (Abt. I) 181:446.

Schoenholz, W. K.: 1962. Experimental studies on lethal and immune mechanisms in massive Bedsonia infection. Thesis, University of California, 191 pp.

———: 1964a. Beitrage zur Pathogenese der Bedsonieninfektion bei Mausen. I. Untersuchungen über den Einfluß der Keimzahl auf die Absterberate. Band 148, Heft 2. pp. 98–107.

———: 1964b. Beitrage zur Pathogenese der Bedsonieninfektion bei Mäusen II. Hamatologische und blutchemische Studien. Arch. f. Hygiene und Bakteriologie, Band 148, Heft 3, pp. 222–28.

Schoop, G., and Kauker, E.: 1955. Tauben als Infektionsquelle eines menschlichen Ornithosefalles. Monatsheft. Tierheilk. 7:120.

Scruggs, J. H.: 1957. Psittacosis. Pub. Health Rep. 72:473.

Semple, A. B.: 1956. Psittacosis. Medical Office of Health, Liverpool, Ann. Rep., pp. 65–66.

Serý, V.: 1962. Personal communication to the author.

———, and Strauss, J.: 1957. Výskyt ornithosy a salmonellosy u racka chechtavého (Larus ridibundus L.). I. Epidemiologická vyzsrovlani. (The incidence of ornithosis and salmonellosis in the black-headed gull (Larus ridibundus L.). I. Epidemiological investigation) Česk. Epidem., Mikrobiol. Immunol. 6:152.

———, Strauss, J., Fantová, Z., Mazel, J., and Vondrášek, V.: 1960. Immunological survey of ornithosis in the population of the Czech regions. Internat. Epidem. Symp., Prague, 22–26 February 1960, 6 manco. pp. In English.

———, Strauss, J., Fantová, Z., Mazel, J., and Vondrásek, V.: 1960. Immunological survey of ornithosis in the population of the Czech regions. Jour. Hyg. Epidem. (Praha) 5:459.

———, Strauss, J., Fric, M., and Kleinbauer, V.: 1957. Epidemie ornithosy ve východních Čechách. (An epidemic of ornithosis in East Bohemia.) Česk. Epidem., Mikrobiol., Immunol. 6.24.

———, Strauss, P., Kříhové, J., Vršiné, M., and Mikešové, P. M.: 1960. Ornithosis and salmonellosis in wild birds in a nature reservation and in poultry and human beings in adjacent surroundings. Veterin. Med. 33:799.

Sever, J. L.: 1962. Application of a microtechnique to viral serological investigations. Jour. Immunol. 88:320.

Shaughnessy, H. J.: 1955. Psittacosis in wild pigeons. In: Psittacosis. Diagnosis, Epidemiology and Control, ed. by F. R. Beaudette. Rutgers University Press, New Jersey, pp. 90–98.

Shimizu, Y., and Bankowski, R. A. 1963a. The characteristics of a bacterium used in complement fixation tests for ornithosis. Avian Dis. 7:331.

———, and Bankowski, R. A.: 1963b. The nature of the cross reactions between a bacterium of the genus Herellea and the ornithosis virus in complement fixation. Am. Jour. Vet. Res. 24:1283.

Shipkowitz, N. L., Meyer, K. F., and Eddie, B.: 1958. Unpublished data on the treatment of pigeon ornithosis.

Shone, D K.: 1953. An outbreak of psittacosis in pigeons Jour. South Afr. Vet. Med. Assn. 24:173.

Siegmund, J.: 1960. Die zunehmende klinische und epidemiologische Bedeutung der Ornithose in der DDR. Zeitschr. Ges. Inn. Med. 15.622.

Smadel, J. E.: 1943. Atypical pneumonia and psittacosis. Jour. Clin. Invest 22.57.

———, Jackson, E. B., and Harman, J. W.: 1945. A new virus disease of pigeons. I. Recovery of the virus. Jour. Exper. Med. 81:385.

———, Wall, M. J., and Gregg, A.: 1945a. An outbreak of psittacosis in pigeons. involving production of inclusion bodies, and transfer of the disease to man Jour. Exper. Med. 78:189.

Smadel, J. E., Wertman, K., and Reagan, R. L.: 1945b. Yolk sac complement fixation antigen for use in psittacosis lymphogranuloma venereum group of diseases. Proc. Soc. Exper. Biol. and Med. 51:70.

Smith, K. O., and Manire, G. P.: 1959. Enumeration of meningopneumonitis virus particles by phase contrast microscopy. Proc. Soc. Exper. Biol. and Med. 100.543.

Solt, K., Domok, I., and Boldsei, T.: 1962/63. Epidemiological and serological analysis of the first ornithosis epidemics in Hungary. Acta Microbiol. Ac. Sci. Hungaricae 9:369.

Spendlove, J. C.: 1957. Production of bacterial aerosols in a rendering plant process. Pub. Health Rep. 72:176.

Stamp, J. T.: 1951. Developmental forms of the virus of ovine enzootic abortion. Jour. Comp. Path. 61:215.

———, McEwen, A. D., Watt, J. A. A., and Nisbet, D. I.: 1950. Enzootic abortion in ewes. I. Transmission of the disease. Vet. Record 62:251.

Steele, J. A., and Scruggs, J. I.: 1958. The epidemiology of psittacosis, 1951–1956. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette, Rutgers University Press, New Brunswick, New Jersey, pp. 32–51.

Strauss, J.: 1956. (Virological examination of ornithosis in men and ducks in Czechoslovakia.) Cesk. Epidem., Mikrobiol, Immunol. 5:281.

———: 1957. Ornithosis in Czechoslovakia. Acta Virol. 1:132.

———: 1961. Soucasná problematika nákaz vyvolaných víry ze skupiny psittacosis ornithosis-lymphogranuloma venereum. I. Problematika virologická. II. Problematika epidemiologická Cas lck cesk, 100:187.

———, Bednár, B., and Serý, V.: 1957. (The incidence of ornithosis and salmonellosis in the black-headed gull (Larus ridibundus L.) I. Epidemiological investigations.) Cesk. Epidem. Mikrobiol., Immunol. 6:152. (II. Isolation and identification of ornithosis virus in the gull with simultaneous detection of Salmonella typhimurium.) Cesk. Epidem., Mikrobiol, Immunol 6:231.

———, Frič, M., and Suková, M.: 1958. Experimentální zkušenosti s přímou a inhibiční reakcí vazby komplementu a jejich použití v diagnostice ornithozy. (Experimental experiences with the direct and the inhibitory (indirect) complement fixation tests and their use in the diagnosis of ornithosis) Cesk. Epidem., Mikrobiol., Immunol. 7:15.

———, and Reisterer, J.: 1960. Ornitóza na východném slovensku. Izolace a identifikace kmenů ornitózy z lidí a kachen. (Ornithosis in eastern Slovakia. Isolation and identification of ornithosis strains in men and ducks) Cesk Epidem., Mikrobiol, Immunol. 9:165.

———, Reisterer, J. and Roztocil, D.: 1960. Ornitóza na východním slovensku. Epidemiologická a serologická problému v Prešovském kraji. (Ornithosis in eastern Slovakia. Epidemiological and serological research in the region of Presov) Prak lck. 40.884.

———, and Serý, V.: 1961. Ornithose in der CSSR. Epidemiologisch-virologische Aspekte. Archiv fur Exper Veterinärmed 18 61.

———, and Skařili, F.: 1962. (Neutralizing antibodies of the globulin fractions after ornithosis.) J. Hyg Epidem. 6.169.

———, Šmejkal, F.: 1959 Persistence viru ornitozy pri hodnocení jeho citlivosti k penicilinu z chloretracyklinu (aureomykinu) a bílých myší. (Persistence of ornithosis virus in studies on its resistance to penicillin and chlortetracycline (aureomycin) in white mice.) Cesk. Epidem, Mikrobiol., Immunol 8 73

———, Šmejkal, F., Vondráček, V., and Kořínku, Z. 1961. Skrmování chlortetracyklinu (aureomykonnu) u kachen s latentní ornitózou. (Feeding of chlortetracycline (aureomycin) to ducks with latent ornithosis.) Vetranární medicína 6 607.

———, and Veselá, B.: 1961. Pokusy o ovlivnění vnímavosti experimentálních zvírat vůči víru ornitózy. (An attempt to influence the sensitivity of laboratory animals to ornithosis virus) Cesk Epidem., Mikrobiol., Immunol 10 92.

Sulkin, S. E., and Pike, R. M.: 1949. Viral infections contracted in the laboratory. New England Jour Med 241 205

Swann, R. H A: 1955 A microscopical study of the reproduction of psittacosis virus. Brit. Jour. Exper. Path 36 507

Tajima, M., Nomura, Y., and Kubota, Y 1957. Structure and development of viruses of the psittacosis lymphogranuloma group observed in the electron microscope. Jour. Bact. 74.605.

Tendeiro, J., Palmeiro, J. M., and Araujo, J: 1949. A ornitose dos columbideos em Portugal. Rev. Med. Vet. Lisboa 44 8

Terhaag, L.: 1956. Über die Bedeutung der Tauben in der Epidemiologie der Ornithose. Arch. Hyg. u Bakt. 140.529.

Terskikh, I. I.: 1954. Ornitoz cheloveka. (Ornithosis in man) Zh. Mikrobiol, Epidemiol. i Immunobiol., pp. 42-50

———: 1956 Contributions to the etiology and epidemiology of ornithosis (psittacosis). Zh. Mikrobiol, Epidemiol. I Immunobiol. I.69.

———: 1957a. Early intradermal diagnosis of ornithosis. Acta Virol. 1:211.

———: 1957b. (Ornithosis in U.S.S.R.) Thesis, Moscow

——: 1962. (Agglutination reaction of elementary bodies in ornithosis virus) Vop. Virusol. 7:215.

——: 1964. Epidemiologie der Ornithose in der UdSSR. (Mit 2 Abbildungen.) Archiv für Exper. Veterinärmed. 18:19.

——, Chel'tsov-Bebutov, A. M., Kuborina, L. N., and Keleinikov, A. A.: 1961. (Studies on ornithosis in birds and on its focal distribution.) Vop Virusol. 6:131.

Chervonsky, V. I., Kareva, M. P., Dormidontov, R. V., Gromyko, A. I., Obukhovskaya, N. M., Kozlyakova, A. I., and Tanukhhova, E. B.: 1962. (Natural and secondary ornithosis focus in Zavidovsky District, Kalinin Oblast.) Vop. Virusol 6:93.

Terzin, A. L.: 1958. Psittacosis-ornithosis-mammalian pneumonia (POMP) viruses in man, mammals and birds. Jour. Hyg. Epidemiol., Microbiol. and Immunol. 2:129.

—— 1960. Different types of serologic reactivity to Bedsonia (psittacosis group) antigen in various hosts. Discussion of some related problems. Jour. Immunol. 85:90.

——, Fornazaric, M. R., and Hlača, D. M.: 1957. Preliminary report on psittacosis ornithosis in Yugoslavia. Acta Virol. 1:203.

——, Gaon, J., Hadžić, M., Harlac, H., and Hlača, V.: 1956. Some viral and rickettsial infections in Bosnia and Herzegovina. A sero epidemiological study. Bul World Health Organ. 15:299.

——, Matuka, S., Fornazarič, M. R., and Hlača, D. M.: 1961. Preparation of group-specific Bedsonia antigens for use in complement-fixation reactions. Acta Virol. 5:78.

Thamm, H.: 1964. Die Sammlungsarbeiten bei Ornithose vom Standpunkt des Veterinärmediziners. Archiv für Exper Veterinärmed. 18:229.

Trojan, J. A., and Strauss, J.: 1955. (An outbreak of ornithosis among workers in a poultry processing combine and experimental proof of its viral etiology.) Cas. lék Cesk. 94:423.

Ulstrup, J. C., and Ness, Chr.: 1957. The prevalence of ornithosis antibodies among pigeons in Oslo. Jour. Oslo City Hospitals 7:33.

United States Department of Agriculture, Agricultural Marketing Service, Poultry Division. 1961. Regulations governing the inspection of poultry and poultry products. Sect. 81, 75 1961, August 1, p. 21.

Vaag, A.: 1959. Ornithosens dødelighed. (Fatal cases of ornithosis.) Ugeskr. f. læger 121:308.

van Vloten, J. G. G.: 1954. Ornithosis bij duiven. Tijdschr. Diergeneesk. 79.695

——— 1959 Ornithosis bij duiven (de behandeling). (Sueptomycin treatment of psittacosis in pigeons) Tijdschr. Diergeneesk. 84:935.

Varella, G.: 1955. Ornitosi-psitacosis en las palomas (Columba livia domestica) de México, D. F. Rev. Inst. salub. enferm. trop., Méx 15:221.

Varnai, G, Derzsy, D., and Szecsenyi, I.: 1960 (Ornithosis infection causing pneumonia) Orv. Betil. 101:1354. (In Hungarian.)

Voigt, A., Hille, G., and Wolfram, G.: 1962. Die Ornithose des Schlachtgeflugels. Monatschr. f. Veterinärmed. 17:169.

Völkert, M., and Christensen, P. M.: 1954. Ornithose (psittacose) i Danmark. Foreløbig meddelelse. (Ornithosis (psittacosis) in Denmark. Preliminary report) Ugeskr. f. læger 116 867.

——, and Christensen, P. M.: 1955. Studies on ornithosis in Denmark. Danish Med. Bul. 2 53.

——, and Christensen, P. M.: 1958. Ornithosis complement-fixation antigens from infected yolk sacs. In: Progress in Psittacosis Research and Control, ed. by F. R. Beaudette. Rutgers University Press, New Brunswick, New Jersey, pp. 117–26.

——, and Matthiesen, M.: 1956. An ornithosis related antigen from a coccoid bacterium. Acta Path. et Microbiol. Scandinav. 39:117.

Wagner, J., Mecklejohn, G., Klingeland, E. J., and Hickish, H. W: 1946. Psittacosis vaccines prepared from chick embryo tissues. Jour. Immunol. 54:35.

——, and Victor, J.: 1953. Psittacosic lesions and respiratory immunity in intradermally vaccinated guinea pigs. Am. Jour Path 29.155.

Walz, A.: 1963. Morphologische Studien zu einem Psittacose-Stamm und seine Veränderungen unter dem Einfluss von Penicillin. I-III. Zentralbl. f. Bakt., I, Orig. 188:29.

Ward, C. G., and Birge, J. P.: 1952. Psittacosis (ornithosis) following contact with pheasants. Report of a case Jour Am. Med. Assn. 150:217.

——, Hitslinger, A. L., Morrissey, R. A., and Birge, J. F.: 1954. Psittacosis-lymphogranuloma venereum virus antibodies in man. Experiences with thirty-seven persons with respiratory disease having significantly high antibody titers. Jour. Am. Med. Assn. 155.1146.

Weiss, E.: 1950. The effect of antibiotics on agents of the psittacosis-lymphogranuloma group. I. The effect of penicillin. Jour. Infect. Dis. 87:209.

——— 1955. The nature of the psittacosis-lymphogranuloma group of microorganisms. Ann. Rev. Microbiol. 9.227.

Wenner, H. A.: 1958. Psittacosis-lymphogranuloma group Advances in Virus Res. 5:39

Westwood, J. C. N.: 1953. Psittacosis: recent epidemiological observations. Proc. Roy. Soc. Med. 46.814.

Weyer, F.: 1964. Weitere Beobachtungen im Rahmen von diagnostischen Tierversuchen bei Ornithose-Psittakose mit Bemerkungen über die Entwicklung der Ornithose-Situation in Deutschland während der letzten Jahre. Zentralbl. f. Bakt., I, Orig. 193:147.

Weyer, F., and Lippelt, H.: 1956. Ein Beitrag zur Frage der Tauben-Ornithose in Deutschland. Zeitschr. f. Hyg. u. Infektionskr. 143:223.

Whitney, E., and Gnesh, G. M.: 1954. Potent psittacosis antigens free of anticomplementary activity. Proc. Soc. Exper. Biol. and Med. 87:356.

Wohlrab, R.: 1955. Die Ornithose und ihre Bedeutung fur die ein heimische Epidemiologie. Desinfek. u. Gesundh. 47:1.

Wolff, F.: 1961. Die Ornithose in den Geflügelfarmen und Geflügel-Schlachtbetrieben der DDR. Hyg. auf dem Lande 2 123.

——.: 1961. *Die Ornithose vom Standpunkt des Arbeitshygienikers. (Mit einer Abbildung)* Archiv für Exper. Veterinärmed. 18.219.

Wolins, W.: 1948. Ornithosis (psittacosis), a review. With a report of eight cases resulting from contact with the domestic Pekin duck. Am. Jour. Med. Sci. 216:551.

Yanamura, H. Y., and Meyer, K. F.: 1941. Studies on the virus of psittacosis cultivated in vitro. Jour. Infect. Dis. 68:1.

——, and Meyer, K. F.: 1942. Acute immunity to Microbacterium multiforme psittacosis in the mouse. Jour. Immunol 44.195.

Yow, E. M., Brennan, J. C., Preston, J., and Levy, S.: 1959. The pathology of psittacosis. Am. Jour. Med. 27:739.

Zahler, S. A., and Moulder, J. A.: 1953. The incorporation of radioactive phosphate into feline pneumonitis virus in the thick embryo yolk sac. Jour. Infect. Dis. 93:159.

Zelenková, L., and Strauss, J.: 1963. (Fluorescent antibodies in the diagnosis of psittacosis.) Cesk Epidem. Mikrobiol., Immunol. 12 140. Abstract: Vet. Bul., 1963, 33:683.

Zichis, J., Shaughnessy, H. J., and Lemke, C.: 1946. Isolation of psittacosis-like viruses from Chicago pigeons. Jour. Bact. 51.616.

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24

Avian Encephalomyelitis
(Epidemic Tremor)

Avian encephalomyelitis (AE) is a viral infection which affects primarily young chickens and is characterized by ataxia and tremor, especially of the head and neck. Jones (1932, 1934) first described and designated this disease as epidemic tremor. However, later the name infectious avian encephalomyelitis was given to this malady by Van Roekel *et al.* (1938, 1939). In 1939 the binomial, avian encephalomyelitis, was recommended by the Committee on Poultry Disease Nomenclature of the American Veterinary Medical Association (Beach, 1939).

HISTORY AND DISTRIBUTION

Jones (1932, 1934) first encountered the disease in May, 1930, in 2-week-old Rhode Island Red chicks received from a commercial flock. Only tremor, but no ataxia, was observed in this outbreak. In April, 1931, a second outbreak was observed in 1-week- and 4-week-old chicks on two different farms, but originating from the

same breeding flock. Tremor and ataxia were noted in these two outbreaks. During the ensuing two years, the author saw an increasing number of outbreaks which occurred in Connecticut, Maine, Massachusetts, and New Hampshire. Since then, an increasing incidence of the disease has been reported by others from widely separated areas.

Recent surveys in this and other countries reveal that the distribution of the disease, while limited, presents an interesting geographical picture. Responses to the survey revealed that the disease had been observed in Australia (Seddon, 1952), Canada (Anon., 1958), England (Markson and Blaxland, 1955), Japan (Kuba, 1964), Korea (Lee, 1958), Scotland (Wilson, 1958), Sweden (Lindgren *et al.*, 1957), and Union of South Africa (Coles, 1956). The geographical distribution of this disease has been reported on every continent in the world. Information obtained from the Animal Health Yearbook, Food and Agri-

culture Organization of the United Nations, reveals that there are countries on each continent where the disease is either suspected or has been confirmed (Animal Health Branch, 1963). This is not surprising since many countries in the various parts of the world have received stock from the United States where the disease is widespread. The virus may be egg borne and hence disease may be disseminated via the egg or through young chicks. Its behavior is very similar wherever it occurs and may be a serious economic problem in the broiler industry, hatcheries, and in certain breeding establishments. Its sudden appearance, often with great severity and unpredictable duration, are reasons for great concern on the part of the breeder, hatcheryman, and broiler producer. While not a major threat to the industry at large, it may be of great economic significance to those individuals who experience the disease in their flocks. Adjustments for chick losses from the disease have been very costly to hatcherymen and hatching-egg producers.

ETIOLOGY

Jones (1934) was the first to propagate successfully the causative agent of the disease in susceptible chicks by intracerebral (IC) inoculation with brain material from spontaneous cases. Experimental infections in chicks revealed signs of the disease 6 to 44 days, with an average of 12 to 28 days, after intracerebral inoculation. The disease was produced more readily upon increased serial passage of the agent in the chicks. Similar observations have been reported by other workers (Olitsky, 1939; Van Roekel et al., 1938; Jungherr, 1939). Jones (1934) demonstrated that the agent was filterable through Seitz and Berkefeld N filters and that the virus in brain tissue could be preserved in 50 per cent glycerin for at least 69 days. Van Roekel et al. (1938) confirmed the filterability of the agent through Seitz and celloidin filters, and Olitsky (1939) reported the agent capable of passing through Seitz 1 and 2 disc filters and

Berkefeld V and N candles. Later, by gradocol membrane filtration, the virus was reported to have a diameter of 20 to 30 mμ (Olitsky and Bauer, 1939). In sedimentation of the virus through centrifugation, Jungherr and Minard (1942) were successful in obtaining a noninfective supernate at 20,000 r.p.m., while the sediment induced infection. Olitsky (1939) was unsuccessful in demonstrating complete sedimentation of the virulent virus after angle centrifugation at 5,400 and 12,000 r.p.m. for one hour.

Fresh hatching eggs and embryonating eggs, 5 to 17 days old, inoculated with virus brain suspensions by various routes, have produced chicks with signs of the disease (Van Roekel et al., 1939, 1941). Among 807 fresh hatching eggs inoculated in nine different trials, and then incubated, only 149 chicks hatched, of which 71 showed signs of AE, some as early as on the day of hatching. Among 370 embryonating eggs inoculated at 10 to 11 days of age, 206 chicks hatched and 44 manifested signs of the disease. These observations were confirmed in part by Jungherr and Minard (1942), but they reported that inoculation of the agent via the chorio-allantoic route failed to produce evidence of the disease in the hatched chicks. Likewise, Kligler and Olitsky (1940) could not demonstrate the virus in the allantois 4 days after inoculation. On the contrary, Feibel et al. (1952) were able to demonstrate the presence of the virus in the allantoic fluid in dead and live embryos 7 days post inoculation.

Recently, the successful propagation of the virus in embryonating eggs via intraocular, allantoic cavity, and yolk sac routes has been reported (Jungherr et al., 1956; Wills and Moulthrop, 1956; Sumner et al., 1957a). The behavior of this virus in chicken embryos will be described under pathogenesis.

Multiplication of the virus in Maitland type cultures of minced whole embryo tissue in vitro in the presence of chick serum has been reported by Kligler and Olitsky (1940). Virus growth was not de-

tected in this medium when embryo brain was substituted for whole embryo tissue. Propagation of the virus in tissue culture has not been reported.

Olitsky (1939) also investigated the behavior of the virus of eastern equine encephalomyelitis in chickens and found that it differed from AE virus.

PATHOGENESIS

Primary isolation of the virus by chick inoculation may yield variable or negative results even though brain material is used as inoculum via the IC route. Jones (1934) inoculated 91 chickens IC with brain and spinal cord suspensions and obtained positive evidence of the disease in only 5 birds, 3 developing symptoms and 2 manifesting only lesions. She concluded that the infective agent was present in the brains in only a small proportion of the chicks selected for the inoculum. Jungherr and Minard (1942) found that among 19 spontaneous cases of AE, 11 takes were obtained in inoculated chicks on first passage and only 5 could be passaged regularly. The percentage of takes on first passage varied from 53 to 100 with an average of 41. The incubation period varied from 18 to 70 days with an average of 28 days, and the titers of the strains ranged from 10^{-2} to 10^{-3}. These workers also stated that the virus concentrations in original material were either low- or sub-infective. However, these viewpoints may not be entirely valid in the light of more recent knowledge that the stock used for inoculation may have had either a passive or active immunity (Sumner et al., 1957a, 1957b).

Van Roekel et al. (1938) noted that in a series of 42 passage experiments with a composite of 2 field strains of virus, the percentage of takes increased from 59 to 88 and the incubation period was shortened from 23 to 9 days. Also the period from first symptom to death decreased with increased serial passage of the virus. It is quite evident that primary isolation of the virus by IC chick inoculation may fail to produce signs or lesions of the disease, but

as the virus becomes adapted in the chick, the disease is readily reproduced even with a decimal dilution of infective brain suspensions as high as 10^{-6} (Olitsky, 1939).

The intracranial route of inoculation has given the most consistent results in reproducing the disease in chickens. Other routes by which the infection has been established in the chicken are the intraperitoneal, subcutaneous, intradermal, intravenous, intramuscular, intrasciatic, intraocular, and intranasal (Olitsky, 1939; Van Roekel et al., 1939; Jungherr and Minard, 1942; Feibel et al., 1952; Schaaf and Lamoreux, 1955). Unsuccessful trials to reproduce the disease via the oral route have been reported by Jones (1934) and Olitsky (1939).

The brain and spinal cord are the most promising sources for virus isolation, although other tissues and organs produced evidence of the disease when inoculated into chicks (Jungherr and Minard, 1942; Van Roekel et al., 1938).

The isolation of the virus from other tissues of the body, aside from the brain and cord, is logical to expect since histopathological evidence of the disease is seen in many tissue sites in spontaneous cases. Jungherr and Minard (1942) observed that feces from adult AE affected flocks contained a filterable factor which is capable of producing brain lesions which were indistinguishable from spontaneous AE virus infection. Feibel et al. (1952) were able to demonstrate the presence of virus in allantoic fluid in embryonating eggs 7 days post inoculation, but were unsuccessful in producing a death pattern after at least 6 "blind" serial passages.

More recently, successful propagation of the virus in embryonating eggs, inoculated via the allantoic and intraocular routes, has been accomplished (Jungherr et al., 1956; Sumner et al., 1957a). Virus passed by the intraocular route produced a titer of 10^{-6} after 11 serial passages; whereas, the allantoic route passed virus attained a titer of 10^{-2} after 9 passages. Wills and Moulthrop (1956) were also successful in propagating the virus in embryos using

FIG. 24.1 – Avian encephalomyelitis, 19-day-old embryos: (left) uninoculated, normal; (right) inoculated, shows a marked stunting and muscular dystrophy.
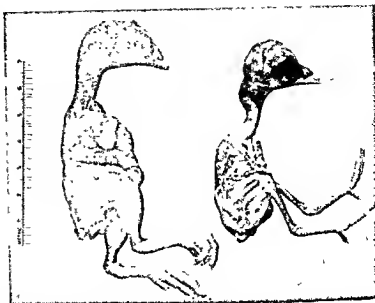
the yolk sac route of inoculation. This route of inoculation was adopted later by other investigators (Sumner et al., 1957b; Calnek and Jehnich, 1959a; Moore and Flowers, 1959; Taylor and Schelling, 1960). The advantages of embryo over chick propagation of the virus have been emphasized, which should greatly facilitate the investigations concerning the disease.

The salient signs and lesions observed in AE embryos are decreased movement and occasionally retardation of growth. Live infected embryos examined at 20 days of age may show a persistent heart beat, but the voluntary muscles would be partially or completely immobilized (Jungherr et al., 1956) (Fig. 24.1) Calnek and Jehnich (1959a) made similar observations concerning the behavior of the virus in embryos. The isolation and propagation of the virus in eggs selected from susceptible flocks is recommended. It also has been emphasized that the isolation and propagation of a field virus be passed in susceptible chicks before attempting to adapt it to susceptible embryos (Poultry Disease Subcommittee, 1963). Calnek and Jehnich (1959a) found that the virus could be detected in the brains of inoculated

embryos 2 to 4 days post inoculation and peak titers (log 5.7 to 6.1) were found 6 to 8 days post infection. The histopathology in the embryo revealed changes that were uniform in character but variable in intensity and location and consisted of encephalomalacia and muscular dystrophy. Among 48 embryos inoculated, 45 exhibited lesions in the central nervous system and 43 showed lesions in the skeletal muscles. The neural lesions were characterized by severe local edema, gliosis, vascular proliferation, and pyknosis. The muscular changes consisted primarily of eosinophilic swelling and necrosis, fragmentation and loss of striations of affected fibers with rare sarcolemmal proliferation and heterophil infiltration (Jungherr et al., 1956).

EPIZOOTIOLOGY

The disease may be observed in all seasons of the year, although it has been suggested that a minor seasonal incidence may occur in November and December and a more pronounced increase from January to June (Jungherr and Minard, 1942). The time and frequency of occurrence of the disease presents a challenging question as to the source of the infection.

In young chicks the disease can nearly always be traced to susceptible parent stock that has experienced a recent infection. In such parent stock one may find, in retrospect, a decline in egg production that usually is transient and may or may not be marked. Taylor and Schelling (1960) reported that susceptible breeding flocks which exist at 5 months of age that some may not encounter the infection for a period of 13 months. At 5 months of age approximately 56.8 per cent of the flocks were immune and at 13 to 18 months of age 95.7 per cent were resistant to the virus based on the embryo susceptibility test. This means that as mature breeding flocks become infected, the virus may be eliminated in the egg and passed on to the chick. The source and mode of spread of the infection to adult breeding flocks is not understood at present (Jungherr and Minard, 1942; Schaaf and Lamoreux, 1955). Naturally infected chicks up to 7 weeks of age have been reported (Jones, 1934). Chickens of all ages are susceptible to the infection by experimental inoculation (Olitsky, 1939; Van Roekel et al., 1939; Feibel et al., 1952).

Evidence of morbidity of the natural disease has been observed only in young stock, although inapparent infections do occur in mature flocks. The usual morbidity rate has been in the range of 10 to 20 per cent with a maximum limit of 60 per cent (Jungherr and Minard, 1942). A high incidence of the disease has occurred in all breeds and under many types of management. The average mortality rate is around 10 per cent and may exceed 50 per cent (Van Roekel et al., 1938).

Natural transmission of the disease has presented a baffling problem. Earlier investigations failed to demonstrate the disease in progeny hatched from eggs obtained from breeders that survived a natural infection (Jones, 1934; Bottorff et al., 1936; Van Roekel et al., 1941). Considerable field evidence and some experimental results show rather conclusively that the infection is egg borne (Van Roekel et al., 1941; Jungherr and Minard, 1942; Schaaf

and Lamoreux, 1955; Taylor et al., 1955; Calnek et al., 1960).

Transmission of the disease by direct or indirect contact, either with naturally or experimentally infected chickens, has been reported. Calnek et al. (1960) suggest that avian encephalomyelitis is an enteric infection in growing and adult birds. The virus may be eliminated in the droppings in sufficient concentration to infect young susceptible chicks by the oral route. Chicks placed in a battery in a contaminated poultry house became infected but to a much lesser degree than chicks in direct contact with infected chicks. Clinical signs occurred among susceptible chicks in contact with infected chicks 11 days after hatching. With the virus strain used, the minimum incubation period following oral administration of the virus-contaminated feces was 11 days. It was suggested that in the field most of the chicks hatched from susceptible dams may become infected as the result of contact with a few egg-borne infected chicks. Attempts to transmit the infection by aerosol means, using a modified Horsfall unit, yielded negative results. Richey (1962) incriminated a ready-to-lay pullet flock housed in the same building but in a separate pen as the source of the infection for outbreaks that occurred in several susceptible breeding flocks 45 weeks of age. Previous history of the pullet flock revealed that it had experienced an acute outbreak of the disease at 3 weeks of age. It was suggested that a "carrier" existed in the pullet flock. While certain aspects of transmission of the disease have been well established, there are other phases that remain unanswered.

Schaaf and Lamoreux (1955) stated that incubator transmission of the disease does not occur, and Jungherr and Minard (1942) reported that hatchability was not affected. On the contrary, Taylor et al. (1955) reported a high embryo death pattern during the last three days of incubation. The percentage of hatchability declined from 78.6 preinfection level to 59.6 during the clinical stage and in-

creased to 75.4 post infection. Eggs produced just prior to and during the period of depressed egg production showed a decreased hatchability and an increased embryonic mortality during the last three days of incubation. Furthermore, the chicks from the hatch with depressed hatchability were the only chicks to show signs of the disease, while the chicks hatched prior to and after the affected hatch appeared normal. Similar observations have been reported by other workers (Calnek *et al.*, 1960; Richey, 1962).

Calnek *et al.* (1960) demonstrated that transmission of the infection can occur in the incubator. Three groups of susceptible eggs were selected. One group was inoculated at 6 days incubation, a second group was uninoculated, but the 2 groups incubated in the same incubator. A third uninoculated group was hatched in a separate incubator. Upon removal of the chicks from the incubators the 2 groups hatched in the same incubator were distributed in wire batteries as well as the uninoculated control group hatched in a separate incubator. The chicks from the inoculated group manifested signs on the first day of age and by the sixth day 49 of 52 showed clinical evidence of the disease. The chicks from the uninoculated group first manifested signs on the tenth day and 15 of 18 chicks exhibited clinical evidence. The control group of 19 chicks remained negative.

Birds recovered from natural and experimental infection develop antibodies in their serum capable of neutralizing the virus (Olitsky, 1939; Jungherr and Minard, 1942; Feibel *et al.*, 1952; Sumner *et al.*, 1957a; Calnek and Jehnich, 1959a, 1959b; Moore and Flowers, 1959; Taylor and Schelling, 1960). In a flock survey for embryo susceptibility to the virus, Sumner *et al.* (1957b) found a wide range in the titration end points of the AE virus in eggs received from 119 flocks. A majority of the tested flocks had no history of AE but produced embryos resistant to the virus, suggesting a mild infection. It was suggested that in an immune flock not all

hens are immune to a degree measurable by the test. Olitsky and Van Roekel (1952) reported that chicks inoculated with an active virus and which failed to show clinical signs of the disease may not be rendered resistant.

The AE virus appears to have a limited host range, including the chicken, pheasant, and Coturnix quail, which have succumbed to natural infection. Hill and Raymond (1962) were able to produce clinical signs of avian encephalomyelitis in quail chicks 1 to 14 days of age. The experimental chicks were maintained in the same room with the adult breeding quail. Fifteen days after the chicks were inoculated, the adult flock manifested a decline in egg production and hatchability. Clinical evidence and mortality were observed among the chicks hatched from eggs collected during the outbreak of the disease. Ducklings, turkey poults, young pigeons, and guinea fowl have been infected experimentally. Mice, guinea pigs, rabbits, and monkeys were refractory to the virus introduced intracerebrally (Van Roekel *et al.*, 1939, 1940; Olitsky and Van Roekel, 1952; Mathey, 1955).

In view of the limited investigations concerning host susceptibility, it would appear most desirable that further studies be undertaken with the improved methods to evaluate the status of the various animal and avian species concerning avian encephalomyelitis.

SYMPTOMATOLOGY

This disease presents an interesting syndrome which may be characterized as follows:

The disease usually makes its appearance in natural outbreaks when the chicks are 1 to 2 weeks of age. Affected chicks have been observed at the time of hatching. The incubation period of the experimental disease varies from 5 to about 40 days, with an average period of 9 to 21 days. Affected young chicks, as a rule, will show first a slightly dull expression of the eyes which is followed by a progressive ataxia or incoordination of the leg

muscles. This ataxia may be detected readily by exercising the chicks. As the ataxia grows more pronounced, the chicks show an inclination to sit on their haunches. When disturbed, such chicks may move about, exhibiting little control over the speed and movement of their gait, and finally come to rest on their haunches or fall on their sides. Some chicks may refuse to move or they may walk on their hocks and shanks. The dull expression becomes more pronounced, accompanied with a weakened cry or an inability to cry. Tremor of the head and neck may become evident. The frequency and magnitude of the tremor may vary. Exciting or disturbing the chick may bring on the tremor, which may continue over various periods of time and recur at irregular intervals. The ataxic signs, as a rule, appear before the tremor, but in some cases only tremor has been observed or may occur prior to the ataxic signs. Jungherr (1939) states that of the histologically positive field cases, 56.9 per cent showed ataxia, 18.3 per cent showed tremor, and 35 per cent both; 9.2 per cent showed no clinical signs. The ataxia usually progresses until the chick is incapable of moving about, and this stage is followed by inanition,

prostration, and finally death (Figs. 24.2 and 24.3). Chicks with marked ataxia and prostration are frequently trampled upon by their pen mates thus hastening death. Some chicks with definite signs of AE may survive and grow to maturity, and in some instances the signs may disappear completely. Among 83 naturally infected chicks reared at the laboratory, only 24 survived after a period of 8 months. Seven of the 24 survivors developed a blindness as the result of an opacity or bluish discoloration of the lens of one or both eyes (Fig. 24.4). A few progenies from these survivors, as they became mature, developed an eye condition that resembled the condition observed in the naturally infected dams. The gross pathology consisted of an apparent enlargement of the eyeball, a marked opacity of the lens, seemingly fixed pupil, and total blindness in some cases. No characteristic signs of AE were detected in these progenies (Van Roekel *et al.*, 1936, 1937). Somewhat similar observations have been reported by other workers (Peckham, 1957; Bridges and Flowers, 1958).

While the disease has been reported to occur in adult flocks, as substantiated by a decline in egg production, positive sero-

FIG. 24.2 — Avian encephalomyelitis in experimentally inoculated chicks. Chick 1 appears normal; chicks 2, 3, and 4 show various stages of the disease.
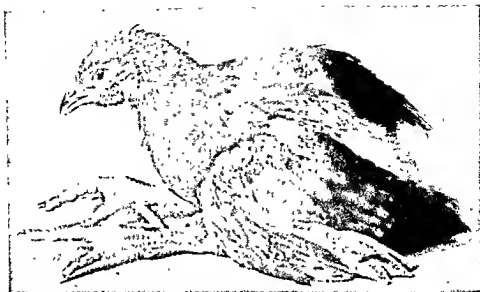
FIG. 24.3 — Avian encephalomyelitis, incubation 13 days, ill 2 days. (Courtesy of P. K. Olitsky, Jour Exper Med.)

logic evidence, and presence of the disease in its progeny, it is of interest that typical signs of AE as seen in chicks do not occur in natural outbreaks in mature birds. Since seminature and mature chickens fail to manifest signs of the disease, one might suspect spread of the virus to occur constantly in a population, and such a process can be detected most readily by serological means.

PATHOLOGY

The lesions are only microscopic. The principal histopathologic changes are distributed among the central nervous system as manifested by gliosis, perivascular infiltrations, neuronal degeneration, and in the viscera as hyperplasia of the lymphoid follicles involving primarily the myocardium, proventricular and ventricular

FIG. 24.4 — A mature pullet having survived an attack of avian encephalomyelitis shows an opacity of the lens.

muscles, and the pancreas (Jones, 1932; Olitsky, 1939; Jungherr and Minard, 1942). No meningeal reaction except a perivascular infiltration of the vessels was observed by Olitsky (1939). Neuronal degeneration was most striking and common among lesions distributed throughout the central nervous system, especially in the pons-medulla and anterior horn cells of the spinal cord in the region of the lumbo-sacral enlargement. The progressive changes consist of an enlargement of the neuron and its nucleus, nuclear eccentricity, tigrolysis, and clearing of the cytoplasm of the Nissl bodies, and in the advanced stages the neuron may disappear completely. In early stages of the infection, there may be occasionally no other sign of involvement of the nervous tissue, especially no inflammatory reaction. The latter, however, is likely to occur in the anterior horns of the cord. Jones (1934) reported the occurrence of severe degeneration of Purkinje cells, while Olitsky (1939) claimed that these cells are usually well preserved and that only here and there some cells show degeneration, especially in the later stages of the disease. Also, in chronic ataxia some indications of neuronophagia and satellitosis may be found. Perivascular reaction may be very pro-

nounced throughout the entire brain, especially in the cortex, pons-medulla, and cerebellum (Figs. 24.5 and 24.6). Perivascular changes were essentially absent in the cord. Demyelinization was not observed, although Jungherr (1939) did note occasionally myelin degeneration in the sciatic nerve. Jungherr and Minard (1942), in a study of 283 spontaneous cases, found the focal lesions in the central nervous system to be comparatively mild and quite independent of the severity of the symptoms.

The visceral lesions consist of hyperplasia of the lymphoid follicles, which are normally quite irregularly distributed throughout the tissues (Fig. 24.7). The hyperplastic follicles are of two types — the one having an irregular outline without a definite boundary, and the other being oval or circular in shape and surrounded by a delicate, capillarized, connective tissue membrane. These focal lesions may be seen in many tissues but seem to have diagnostic significance when they occur in the ventriculus, proventriculus, pancreas, and heart and when accompanied by specific lesions in the central nervous system (Jungherr and Minard, 1942). These same authors also stated that in correlating pathologic evidence with the

FIG. 24.5 – Perivascular lesion in cerebellum and loss of Purkinje cells. Incubation period 14 days; ataxic for 22 days. X125. (Courtesy of P. K. Olitsky, Jour. Exper. Med.)

FIG. 24.6 — Details of perivascular lesion in cerebellum. ×500. (Courtesy of P. K. Olitsky, Jour. Exper. Med.)

symptoms, in spontaneous cases, 11 per cent manifested symptoms but revealed no lesions, and, on the contrary, 8 per cent of the symptomless cases revealed typical lesions.

The pathology of experimental cases was quite similar to that of the spontaneous, except that the perivascular reactions in the central nervous system frequently showed massive development, especially in the cerebrum. Visceral lesions

were much less pronounced and frequently absent.

IMMUNOLOGY

Chickens that have been exposed to the live virus will manifest an immunologic response that can be measured with the standard virus neutralization test (Sumner et al., 1957b; Calnek and Jehnich, 1959a). The Van Roekel embryo-adapted strain is recommended to determine the neutrali-

FIG. 24.7 — Hyperplasia of the lymphoid islands of liver. ×275. (Courtesy of P. K. Olitsky, Jour. Exper. Med.)

ing capacity of the serum or plasma. The undiluted serum or plasma is mixed with tenfold dilutions of the virus and the resulting mixtures are inoculated via the yolk sac into susceptible 6-day-old embryonated chicken eggs. The embryos are examined 10 to 12 days post inoculation for lesions characteristic of the virus. The neutralization index is calculated as the log difference between EID_{50} virus titer and EID_{50} virus-serum titer. A log index of 1.1 or greater is considered as positive evidence of previous exposure to the virus. Among samples from a recently exposed flock, the log index may vary from 1.5 to 3.0. Neutralizing antibodies may be detected as early as the second week after exposure and remain at significant levels for at least several months. Calnek and Jehnich (1959a) reported that in many instances birds having no detectable neutralizing antibodies (NI of less than 1.1) would resist intracerebral challenge with as many as ten thousand EID_{50} of virus.

Another method used to determine the immunity of a flock is the embryo susceptibility test. Sumner et al. (1957b) first employed this test as a means to determine which breeding flocks had experienced the infection. A small sample of fertile eggs is selected from each flock and the eggs are inoculated after 6 days of incubation with the egg-adapted virus via the yolk sac. Ten to 12 days post inoculation the embryos are examined for characteristic lesions of the virus. If 0 to 50 per cent of the embryos show no signs of infection, the flock is considered immune. If more than 50 per cent but less than 100 per cent of the embryos are affected, it strongly suggests a recent exposure to the virus. If all embryos are affected, the flock is susceptible. Adequate controls should be included in conducting the embryo susceptibility test. This test serves as a useful tool under certain circumstances, but it is used to a very limited degree. The availability of susceptible eggs has been a deterrent in the wider usage of this test.

DIAGNOSIS

In spontaneous cases a tentative and frequently a definite diagnosis of disease can be made when a complete history of the flock and typical specimens of the disease are furnished the diagnostician. Histopathologic evidence of gliosis, lymphocytic perivascular infiltration, the axon type of neuronal degeneration in the central nervous system, and the hyperplasia of the lymphoid follicles in certain visceral tissues usually can be considered as a basis for a positive diagnosis. The isolation of the virus or the serum-virus neutralization test gives a more specific diagnostic result. Recently the latter method has been employed more extensively and with further refinements and improvements should furnish a very useful tool for the specific diagnosis of avian encephalomyelitis. Caution should be given that this disease may be confused with other avian diseases manifesting similar clinical signs. Among such diseases may be included Newcastle disease, equine encephalomyelitis infection, nutritional disturbances (rickets, encephalomalacia, and riboflavin deficiency), and avian leukosis complex. Specific diagnostic criteria have been described for these diseases in other chapters of this text.

PREVENTION AND CONTROL

No satisfactory treatment is known for acute outbreaks in young chicks. It is advisable to check the management in order that the chicks will receive adequate nutrition and a suitable environment. The removal and segregation of affected chicks may be indicated under certain conditions, but generally it is advisable to kill affected specimens since they will not develop into profitable stock. If the disease appears frequently in progeny from the same flock, it is advisable to cease hatching from such a flock for a brief period of time.

It appears that once a flock has experienced an outbreak of the disease no fur-

ther evidence of the disease is likely to be observed (Schaaf and Lamoreux, 1955). Natural specific antibodies for the virus have been detected in the sera and in the yolk of fresh eggs obtained from survivors of the disease (Sumner et al., 1957b).

Within recent years effective immunization procedures have been developed to protect breeding flocks against outbreaks of the disease and in turn prevent the spread of the virus to the progeny via the egg-borne route (Schaaf, 1958, 1959; Calnek et al., 1961; Butterfield et al., 1961; Van Roekel et al., 1961). Schaaf (1958) first recognized that chickens exposed to the virus developed an immunity and that such birds should not produce infected progeny. This observation formed the basis for a program of vaccinating growing birds with the wing-web method prior to sexual maturity. Among more than one million chickens vaccinated with the live virus, no evidence of avian encephalomyelitis was observed in progeny produced by these vaccinated birds.

Calnek et al. (1961) were successful in immunizing immature birds by using a satisfactory vaccine strain which was ad-

ministered by the oral route. It was observed that by vaccinating only a small percentage of the birds the remainder of the flock would develop a satisfactory immunity. Since progeny from vaccinated or naturally infected flocks may retain passive antibodies for approximately 8 weeks, they should not be vaccinated until 10 weeks of age or older. It is recommended that all replacement stock for breeding flocks be immunized against the disease.

Effective inactivated vaccines for avian encephalomyelitis have also been produced (Calnek and Jehnich, 1959b; Schaaf, 1959; Butterfield et al., 1961). At the present time the inactivated vaccine may be applicable for susceptible breeding flocks that are in production and for breeding flocks located in areas where the disease incidence is low.

A commercial live virus vaccine, administered in the drinking water, is available at the present time. An effective immunization program applied to all breeding flocks should eliminate clinical outbreaks of the disease in the chick population.

REFERENCES

Animal Health Branch. 1963. Animal Health Yearbook. Food and Agriculture Organization of the United Nations. Printed in Italy. P. 399.

Anonymous. 1958. Report of the committee on nomenclature and reporting of diseases. Proc. 30th Ann. Meet. Northeastern Conf. on Avian Dis., June 30–July 2, Cornell Univ., Ithaca, N.Y.

Beach, J. R. 1939. Poultry diseases. Jour. Am. Vet. Med. Assn. 95.615

Botroff, C. A., Tepper, A. E., Martin, C. L., Charles, T. B., and Reed, F. D. 1936. Epidemic tremors (trembling chick disease). New Hampshire Agr. Exper. Sta. Circ. 51.1.

Bridges, C. H., and Flowers, A. I. 1958. Iridocyclitis and cataracts associated with an encephalomyelitis in chickens. Jour. Am. Vet. Med. Assn. 132.79

Butterfield, W. K., Luginbuhl, R. E., Helmboldt, C. F., and Sumner, F. W. 1961. Studies on avian encephalomyelitis. III. Immunization with an inactivated virus. Avian Dis. 5.445.

Calnek, B. W., and Jehnich, H. 1959a. Studies on avian encephalomyelitis. I. The use of a serum neutralization test in the detection of immunity levels. Avian Dis. 3.95.

——, and Jehnich, H. 1959b. Studies on avian encephalomyelitis. II. Immune responses to vaccination procedures. Avian Dis. 3.225.

——, Taylor, P. J., and Sevoian, M. 1960. Studies on avian encephalomyelitis. IV. Epizootiology. Avian Dis. 4.325.

——, Taylor, P. J. and Sevoian, M. 1961. Studies on avian encephalomyelitis. V. Development and application of an oral vaccine. Avian Dis. 5.297.

Coles, D.J. 1956. Personal communication. Div. Vet. Serv., Dept. Agr., Onderstepoort, Union of So. Africa.

Feibel, F., Helmboldt, C. F., Jungherr, E. L., and Carson, J. R. 1952. Avian encephalomyelitis — prevalence, pathogenicity of the virus, and breed susceptibility. Am. Jour. Vet. Res. 13.260.

Hill, R. W., and Raymond, R. G. 1962. Apparent natural infection of Coturnix quail hens with the virus of avian encephalomyelitis. Case Report. Avian Dis. 6.226.

Jones, E. E. 1932. An encephalomyelitis in the chicken. Science 76.331.

———: 1954. Epidemic tremor, an encephalomyelitis affecting young chickens. Jour. Exper. Med. 59:781.

Jungherr, E. L.: 1939. Pathology of spontaneous and experimental cases of epidemic tremor. Poultry Sci. 18:406.

———, and Minard, E. L.: 1942. The present status of avian encephalomyelitis. Jour. Am. Vet. Med. Assn. 100.38.

———, Sumner, F., and Luginbuhl, R. E.: 1956. Pathology of egg-adapted avian encephalomyelitis. Science 124:80.

Kligler, I. J., and Olitsky, P. K.: 1940. Experiments on the cultivation of virus of infectious avian encephalomyelitis. Proc. Soc. Exper. Biol. and Med. 43.680.

Kuba, N.: 1964. Personal communication. Lab. Vet. Med. Hyogo Univ, Sasayama, Hyogo, Japan.

Lee, C. K.: 1958. Personal communication. Nat. Inst. Vet. Res, Dept. Agr., Pusan, Korea.

Lindgren, N. O., Nilsson, A., and Rakos, K.: 1957. Infektiøse aviare encephalomyelitis beim kücken in Schweden. Nordisk. Vet.-Med. 9:801.

Mathson, I. M., and Blaxland, J. D.: 1955. Suspected infectious avian encephalomyelitis in poultry in Britain. Vet. Record 67 131.

Mathey, W. J., Jr.: 1955. Avian encephalomyelitis in pheasants. Cornell Vet. 45:89.

Moore, R. W., and Flowers, A. I.: 1959. The development of a chicken embryo lethal strain of avian encephalomyelitis virus. Avian Dis. 3:239.

Olitsky, P. K.: 1939. Experimental studies on the virus of infectious avian encephalomyelitis. Jour. Exper. Med. 70.565.

———, and Bauer, J. H.: 1939. Ultrafiltration of the virus of infectious avian encephalomyelitis. Proc. Soc. Exper. Biol. and Med. 42:634.

———, and Van Roekel, H.: 1952. Avian encephalomyelitis (epidemic tremor). In Diseases of Poultry (Biester and Schwarte, eds), 3rd ed. Iowa State University Press, Ames. Pp. 619–28

Peckham, M. C.: 1957. Case report—lens opacities in fowls possibly associated with epidemic tremors. Avian Dis. 1.247.

Poultry Disease Subcommittee: 1963. Avian encephalomyelitis (epidemic tremors). Methods for the Examination of Poultry Biologics, 2nd ed., Pub. 1038. National Research Council, National Academy of Sciences, Washington, D.C. Pp. 87–93.

Richey, D. J.: 1962. Avian encephalomyelitis (epidemic tremor). Case report. Southeastern Vet 13:55.

Schaaf, K.: 1938. Immunization for the control of avian encephalomyelitis. Avian Dis. 2:279.

———: 1959. Avian encephalomyelitis immunization with inactivated virus. Avian Dis. 3:245.

———, and Lamoreux, W. F.: 1955. Control of avian encephalomyelitis by vaccination. Am. Jour. Vet. Res. 16.627.

Seddon, H. R.: 1952. Diseases of domestic animals in Australia. Part 4, protozoan and viral diseases. Serv. Publ. Div. Vet. Hyg.) No. 8. Australia Dept. Health, 147.

Sumner, F. W., Jungherr, E. L., and Luginbuhl, R. E.: 1957a. Studies on avian encephalomyelitis. I. Egg adaptation of the virus. Am. Jour. Vet. Res. 18.717.

———, Luginbuhl, R. E., and Jungherr, E. L.: 1957b. Studies on avian encephalomyelitis. II. Flock survey for embryo susceptibility to the virus. Am. Jour. Vet. Res. 18.720.

Taylor, J. R. E., and Schelling, E. P.: 1960. The distribution of avian encephalomyelitis in North America as indicated by an immunity test. Avian Dis. 4:122.

Taylor, L. W., Lowry, D. C., and Raggi, L. G.: 1955. Effects of an outbreak of avian encephalomyelitis (epidemic tremor) in a breeding flock. Poultry Sci. 34:1036.

Van Roekel, H., Bullis, K. L., and Clarke, M. K.: 1938. Preliminary report on infectious avian encephalomyelitis. Jour. Am. Vet. Med. Assn. 93.372.

———, Bullis, K. L., and Clarke, M. K.: 1939. Infectious avian encephalomyelitis. Vet. Med. 34:754.

———, Bullis, K. L., and Clarke, M. K.: 1941. Transmission of avian encephalomyelitis. Jour. Am. Vet. Med. Assn. 99:220.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1938. "Epidemic tremors" in chickens. Mass. Agr. Exper. Sta. Ann. Rep., Bul. 327:75.

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1937. "Epidemic tremor" in chicks. Mass. Agr. Exper. Sta. Ann. Rep., Bul. 359.89

———, Bullis, K. L., Flint, O. S., and Clarke, M. K.: 1940. Avian encephalomyelitis. Mass. Agr. Exper. Sta. Ann. Rep., Bul. 369.94.

———, Calnek, B. W., Luginbuhl, R. E., and McKercher, P. D.: 1961. Committee report on a tentative program for the control of avian encephalomyelitis. Avian Dis. 5:456.

Wills, F. K., and Moulthrop, I. M.: 1956. Propagation of avian encephalomyelitis virus in the chick embryo. Southwest. Vet. 10.39.

Wilson, J. E.: 1958. Personal communication. Veterinary Laboratory, Lasswade, Midlothian, Scotland.

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25

Equine Encephalomyelitis Virus in Birds

A few years after the viral etiologies of equine encephalomyelitis were established, naturally occurring diseases of birds were shown to be due to eastern equine encephalomyelitis (EEE) virus. Fothergill and Dingle (1938) reported the isolation of this virus from a pigeon submitted by a breeder in Massachusetts during the 1938 epidemic, which involved both man and horses. Simultaneously, ring-necked pheasants in Connecticut were found to be naturally infected with the eastern virus by Tyzzer et al. (1938) During the same year, Van Roekel and Clarke (1939) isolated the eastern virus from a ring-necked pheasant submitted to them by a game breeder in New Jersey. Since that time, recurring epidemics of eastern equine encephalomyelitis have been reported from New Jersey and Connecticut by Beaudette and Hudson (1945), Beaudette and Black (1948), Cohen and Sussman (1957), and Luginbuhl et al. (1958). Western equine encephalomyelitis (WEE) virus has not

been reported as producing clinically evident, naturally occurring disease in domestic poultry or game birds.

Etiology. Two antigenically different viruses cause encephalitis in horses in North America. Because of the geographical distribution of the disease in horses, these are called eastern equine encephalomyelitis (EEE) virus and western equine encephalomyelitis (WEE) virus. These viruses are spherical in shape and are 40 to 50 mμ in diameter. They are readily inactivated by a number of chemical agents such as ether, chlorine, phenol, etc. These viruses are most stable in a menstruum containing 10 per cent or more serum, a low salt concentration, and buffered to pH 7.6 or above. They may be preserved in 50 per cent glycerin buffered to pH 7.6 for short periods or frozen at -70° C. for periods of several years.

These viruses have a wide host range, readily infecting most laboratory animals when inoculated intracerebrally. Mice and

guinea pigs are especially susceptible, dying of encephalitis within 2 to 5 days when inoculated with the eastern virus and in 4 to 7 days when inoculated with the western virus. The eastern virus is more infective for mammals by peripheral routes of inoculation than is the western virus. Both viruses cause death of chicken embryos within 18 to 72 hours after inoculation by any route. The amniotic route of inoculation is more sensitive than other routes. The embryos present a purplish hemorrhagic appearance when death is due to specific virus infection. A number of tissue cultures show cytopathic changes when infected with these viruses. Chicken and duck embryo fibroblast and hamster kidney cultures have received the most widespread use in diagnostic work.

Symptoms. Chickens more than 4 weeks of age show no clinical signs of disease when infected by these viruses. The effect of infection upon egg production is not known. Day-old chicks succumb rapidly to infection by these agents without showing signs of central nervous system involvement (Chamberlain et al., 1954). Signs of disease in young chicks consist of diarrhea and extreme prostration. Histologically, myocarditis is the outstanding lesion (Tyzzer and Sellards, 1941).

Only eastern equine encephalomyelitis virus has been shown to cause clinical disease in ring-necked pheasants. In this species the outstanding signs of infection are referrable to central nervous system involvement and consist of leg paralysis, torticollis, and tremors. No prominent gross lesions are seen upon postmortem examination. The mortality among clinically ill pheasants is approximately 75 per cent. Recovery from the acute phase of the disease may be accompanied by sequelae consisting of incoordination or muscular weakness. Pheasants may also experience clinically inapparent infections with eastern equine encephalomyelitis virus.

Native wild birds usually experience inapparent infections with these viruses (Kissling et al., 1954). Clinical disease and death may result in introduced species such as the English sparrow (Stamm and Kissling, 1956) and domestic pigeon.

Transmission. Both viruses are transmitted among wild birds principally by mosquito vectors. Not all species of mosquitoes are capable of transmitting these viruses. Virus is circulated in the blood of infected birds for periods of 2 to 5 days. Small passerine birds tend to circulate virus in higher concentrations than do larger birds and therefore are potentially more important sources of virus for mosquito infection.

Eastern equine encephalomyelitis virus is transmitted chiefly by Culiseta melanura among the wild bird population under endemic conditions (Chamberlain, 1958). This mosquito is found only in areas of fresh water swamps. Although its biology is not known completely, it is thought to be preferentially a bird feeder. During epidemic periods, other mosquito species such as Mansonia spp. and Aedes spp. may become important transmitters of the virus (Chamberlain et al., 1954). Culex species of the eastern United States are extremely poor transmitters of the eastern virus. Since Culex pipiens and related species are the common mosquitoes found near poultry houses, this may explain the low incidence of antibody to EEE found in chickens.

There is ample evidence that direct transmission of EEE may occur among pheasants during the acts of feather picking and cannibalism. The virus is present in the blood, feather quills, and mouth secretions of infected pheasants. Pheasants may be experimentally infected by oral administration of the virus (Holden, 1955; Satriano et al., 1958).

WEE virus is transmitted chiefly by Culex tarsalis in the western part of the United States. In the eastern United States, WEE virus usually shares the same habitat as EEE virus and is probably transmitted by the same vectors.

These viruses may occasionally be transmitted mechanically by biting insects due to contamination of their mouth parts

with virus. Transmission by mosquitoes depends upon a cycle of virus development within the arthropod, and, once infected, such insects may remain infected for life.

Diagnosis. Since pathological examination reveals no pathognomonic lesions and clinical signs may be confused with Newcastle disease, definitive diagnosis must be made by isolation and identification of the virus.

Although a variety of laboratory animals are suited for isolation of EEE or WEE virus, chicken embryos and freshly hatched chicks are more sensitive indicators of virus than any other host. Embryos 9 to 14 days of age are satisfactory for use. Inoculation should be into the amniotic cavity. Freshly hatched chicks should be less than ½ day of age and are inoculated subcutaneously. In pheasants the brain is the most likely tissue to yield virus, while in other species the liver, spleen, and heart are more likely to contain virus than is the brain. The tissue emulsion may be treated to control bacterial contaminants by the addition of 1,000 units of sodium penicillin G and 2 mg. of streptomycin sulfate per ml. When the inoculum contains virus the embryos should die within 18 to 72 hours after inoculation and present a hemorrhagic appearance.

Specific identification may be made by serological methods, either neutralization or complement fixation (CF). The allantoic fluid from the infected embryos may serve as antigen in either case. In the complement fixation test the allantoic fluid is tested against serum known to fix complement in the presence of EEE antigen. The CF reactivity of the fluid is usually

rather low, and expected titers are generally in the range of 1:2 to 1:8. Appropriate controls to determine anti-complementary and nonspecific reactions are necessary.

For the neutralization test the infected fluid must be diluted to contain 1,000 to 100 embryo LD_{50}, and it is incubated with specific immune serum before inoculation of the test hosts. Infected allantoic fluids usually contain 10^8 to 10^9 LD_{50} per 0.1 ml. Chicken embryos, mice, or tissue cultures may be used as hosts in the neutralization test for identification purposes. Appropriate controls, using normal serum in place of the immune serum, are necessary.

Birds recovering from EEE infection develop specific neutralizing and hemagglutinin inhibiting antibodies against the virus.

Although WEE is not known to cause clinical disease in poultry, the diagnostic procedures are similar to those for EEE.

Prevention. Protection against mosquitoes will prevent the introduction of EEE virus into pheasant pens. Where this is impractical, debeaking or other methods of preventing cannibalism and feather pulling will check the spread of virus should it be introduced by mosquito bite.

Prophylactic immunization using products designed for equine use may lower the mortality rate should the virus be introduced. These vaccines are usually diluted 1:5 for pheasant use.

Where possible, rearing pens for pheasants should be located away from the margins of fresh water swamps, since it is in areas such as these that the virus is found endemically in wild birds.

REFERENCES

Beaudette, F. R., and Black, J. J.: 1948 Equine encephalomyelitis in New Jersey pheasants in 1945 and 1946. Jour. Am. Vet. Med. Assn. 112.140.
——, and Hudson, C. B.: 1945 Additional outbreaks of equine encephalomyelitis in New Jersey pheasants. Jour. Am. Vet. Med. Assn. 107:384.
Chamberlain, R. W.: 1958. Vector relationships of the arthropod borne encephalitides in North America. Ann. N. Y. Acad. Sci. 70.312.
Sikes, R. K., and Kissling, R. E.: 1954. Use of chicks in eastern and western equine encephalitis studies. Jour. Immunol. 73:106.
——, Sikes, R. K., Nelson, D. B., and Sudia, W. D.: 1954. Studies on the North American arthropod-borne encephalitides. VI. Quantitative determinations of virus vector relationships. Am. Jour. Hyg. 60.278.

Cohen, D., and Sussman, O.: 1957. Equine encephalomyelitis in New Jersey. Public Health News *(New Jersey State Department of Health)* 37:220.

Fothergill, L. D., and Dingle, J. H.: 1938. A fatal disease of pigeons caused by the virus of the eastern variety of equine encephalomyelitis. Science 88:549.

Holden, P.: 1955. Transmission of eastern equine encephalomyelitis in ring-necked pheasants. Proc. Soc. Exper. Biol. and Med. 88 607.

Kissling, R. E., Chamberlain, R. W., Sikes, R. K., and Eidson, M. E.: 1954. Studies on the North American arthropod-borne encephalitides. III. Eastern equine encephalitis in wild birds. Am. Jour. Hyg. 60:251.

Lugnibuhl, R. E., Satriano, S. F., Helmboldt, C. F., Lamson, A. L., and Jungherr, E. L.: 1958. Investigation of eastern equine encephalomyelitis. II. Outbreaks in Connecticut pheasants. Am. Jour. Hyg. 67:4.

Satriano, S. F., Lugnbuhl, R. E., Wallis, R. C., Jungherr, E. L., and Williamson, L. A.: 1958 Investigation of eastern equine encephalomyelitis. IV. Susceptibility and transmission studies with virus of pheasant origin. Am. Jour. Hyg. 67.21.

Stamm, D. D., and Kissling, R. E.: 1956. Influence of season on EEE infection in English sparrows. Proc. Soc. Exper. Biol. and Med. 92:374.

Tyzzer, E. E., and Sellards, A. W.: 1941. The pathology of equine encephalomyelitis in young chickens. Am. Jour. Hyg. 33(Sec. B): 69.

———, Sellards, A. W., and Bennett, B. L.: 1938. The occurrence in nature of "equine encephalomyelitis" in the ring-necked pheasant. Science 88.505.

Van Roekel, H., and Clarke, M. K.: 1939. Equine encephalomyelitis virus (eastern type) isolated from ring-necked pheasant. Jour. Am. Vet. Med. Assn. 94·466.

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26

Fowl Pox

Synonyms. Chicken pox, avian pox, bird pox, contagious epithelioma, sorehead, avian molluscum, avian diphtheria, bird pox diphtheria, canker, *Geflügelpocken* (German), *variole aviaire* (French), *viruela aviar* (Spanish), *difteria aviar* (Spanish), *bouba* (Portugese).

Etiology. Fowl pox has been observed in avian species from time immemorial. Excellent reviews are presented by Goodpasture (1928) and by Beaudette (1949, 1953). The causal agent is a filterable virus, as was first demonstrated by Marx and Sticker (1902) Carnwarth (1908) and others showed that the virus is responsible for both the cutaneous and diphtheritic forms of the disease. There appear to be at least four different viruses or strains of virus causing pox among birds. These are classified by van Rooyen (1954) as *Borreliota avium* (fowl pox virus), *Borreliota meleagridis* (turkey pox virus), *Borreliota fringillae* (canary pox virus) and *Borreli-*

ota columbae (pigeon pox virus). *Borreliota avium* is the type species of the viruses of the animal pox group. Each virus is infective for its homologous host and in some instances for heterologous hosts.

The identification of the common etiology of the pox viruses has been attempted by passage of the viruses through homologous and heterologous hosts and using the criteria of immunogenesis and pathogenesis for the interpretation of the results. In many investigations, quantitative determinations of the virus content of the vaccines were not taken into consideration, and it seems obvious that an accurate assessment of the antigenic properties of such preparations would be impossible.

The present knowledge of avian pox does not allow an adequate classification of the pox viruses without reference to host origin. Some avian pox viruses may

be mono-, bi-, or tri-pathogenic with respect to transmission to certain species of birds.

Gallagher (1916) described an outbreak of fowl pox in quail transmissible to chickens. Ward and Gallagher (1920) stated that pox occurs naturally among geese, ducks, and guinea fowl, and that pheasants and various wild birds are also susceptible. Te Hennepe (1926) reported that of 268 ducks examined by him during 1924, 17 were affected with mouth lesions and 14 with cutaneous lesions of fowl pox. Later, te Hennepe (1927) did not observe fowl pox infection in 304 ducks examined during 1925–26. Doyle and Minett (1927) were unable to infect ducks or a seagull with fowl pox virus. Pigeons are generally resistant to infection with fowl pox virus, but Doyle and Minett were able to adapt a strain of the virus to the pigeon by frequent serial passage.

Irons (1934) studied the immunological relationship of several strains of fowl, turkey, and pigeon pox viruses and suggested that some strains may be rendered "bi-pathogenic" by passage through a series of heterologous hosts, e.g., fowl pox virus passaged through pigeons. All the pigeon pox strains studied were found to be infective for chickens. Negative results were obtained in attempts to infect pigeons with turkey virus. Crows, hawks, owls, ducks, guinea fowls, starlings, and several other species were refractory to the fowl and pigeon strains of virus. Chickens were susceptible but pigeons were refractory to turkey virus. One strain of pigeon pox virus proved infectious for the English sparrow and certain related species. After a single passage in chickens the virus of pigeon pox was greatly attenuated for the pigeon. Repeated passage of pigeon pox virus in chickens, with one possible exception, failed to destroy the infectivity of the virus for pigeons. One strain of fowl pox virus was transmissible with gradually increasing virulence in pigeons but was temporarily attenuated for chickens. Two other strains of fowl pox virus were noninfectious for pigeons.

Beach (1939) classified avian pox according to the host—fowl, turkey, pigeon, and canary—with a list of the species in which it occurs or to which it has been transmitted.

Syverton and Cowan (1944) reported the recovery of fowl pox virus from the sooty grouse. They state that although avian pox is reported to have occurred under natural or experimental conditions in a wide variety of birds, more conclusive evidence appears to be limited to the chicken, pigeon, turkey, guinea fowl, canary, partridge, quail, and pheasant.

A natural outbreak of pox in mourning doves transmissible to other mourning doves and a ring dove through cohabitation was reported by Kossack and Hanson (1954) to be caused by pigeon pox virus.

The virus of turkey pox usually has been considered to be the same as that of fowl pox, but the results obtained by some investigators indicate certain strain differences between viruses causing pox in these two species. Brunett (1934) studied the immunological and pathological relationship between a strain of pox virus obtained from a natural case of the disease in turkeys and a strain of fowl pox virus. No differences were observed in the effect of the two viruses on turkeys, chickens, and pigeons except that the disease was of a longer duration in turkeys and chickens inoculated with fowl pox virus, as compared to inoculation with turkey pox virus. Immunity studies showed that turkeys and chickens inoculated with turkey pox virus or fowl pox virus were immune to subsequent inoculations with these viruses. Pigeon pox virus produced a severe reaction on turkeys, but it failed to produce any immunity to turkey or fowl pox viruses.

Brandly and Dunlap (1938) inoculated chickens with a strain of pox virus obtained from a natural case of the disease in turkeys and observed only a mild cutaneous reaction. No infection was produced at the fifth passage of the virus in chickens.

Beaudette and Hudson (1941) reported

that a strain of turkey pox virus studied by them produced a more severe local and systemic reaction and a higher percentage of secondary head lesions in chickens than fowl pox virus. The lesions consisted of a typical scab of considerable thickness as compared to the atypical scab formation and decreased virulence of the turkey strain studied by Brandly and Dunlap (1938). The turkey virus was less lethal for embryonating chicken eggs than was the fowl pox strain. In cross-immunity tests, Brandly and Dunlap used canary, pigeon, turkey, and fowl pox viruses for a study of the immunological relationships of these viruses and concluded ".. that canary virus immunizes against itself and against pigeon virus to a high degree. The canary virus seems to produce no immunity against turkey virus but apparently a slight immunity to the fowl virus. Similarly, pigeon virus protects against itself and canary virus but does not give complete protection against turkey and fowl viruses. Turkey and fowl viruses give almost complete protection against the four viruses used."

Kikuth and Gollub (1932) found in their work on bird malaria a virus capable of producing a highly fatal disease of canaries. This virus has been the subject of controversy concerning its relationship to the avian pox viruses.

Burnet (1933a) concluded that Kikuth's virus was a member of the bird pox group. The virus was not pathogenic for day-old chicks, half-grown fowls, pigeons, and parrots (budgerigars). Sparrows were susceptible to infection.

Burnet and Lush (1936) showed by the embryonating chicken egg technique that fowl pox and Kikuth's strain of canary pox, although not identical, are serologically related. They stated that "if canary-pox and fowl-pox are serologically almost identical, it is more than likely that all bird-pox strains are similarly related."

Burnet (1933b) was able to infect canaries with pox material collected from a spontaneous outbreak in wild sparrows. The disease in the canaries was similar to that produced by Kikuth's canary virus, but the difficulties in filtration of the virus and the agglutinative tendencies of the virus particles resembled fowl pox virus

McGaughey and Burnet (1945) studied three cases of avian pox in wild sparrows and concluded that the virus resembled canary pox in causing a fatal disease in sparrows and canaries and localized lesions in fowls and pigeons.

The strain of canary pox virus studied by Reis and Nobrega (1937) was "tri-pathogenic" as it infected chickens, pigeons, and canaries. The virus did not dissociate into mono-pathogenic strains after serial passage through chicks, pigeons, and canaries. Typical inclusion bodies were found in the infected birds, and those which recovered developed immunity against the homologous virus as well as fowl and pigeon pox viruses. Birds vaccinated with either fowl pox or pigeon pox viruses developed immunity against the canary pox strain. The canaries from which the strain was isolated suffered from a severe pox disease in which the mortality was 98 per cent.

Grosso and Prieto (1939) have reported a "tri-pathogenic" canary virus which produced only a mild, transitory, localized lesion in chicks and pigeons.

Antoniotti and Romat (1940) studied a canary pox virus which was pathogenic only for canaries.

Durant and McDougle (1938) conducted studies of the immunological relationship of canary pox virus to fowl pox virus with the following conclusions: "Chickens, turkeys, and quail when inoculated with canary pox virus, though fairly typical gross lesions are produced, later when inoculated with fowl-pox virus will develop typical lesions, indicating that no immunity to the fowl-pox virus had been developed by exposure to the canary-pox. This would seem to indicate that canary pox virus is a different type of virus with a different degree of virulence for other bird species than the canary."

Coulston and Manwell (1941) reported that canary pox virus was found to be in-

fective for English and song sparrows but not for cowbirds, starlings, or chickens. Canaries which had been vaccinated with the virus attenuated through long storage did develop an immunity which protected them to some degree, but not completely, against exposure to fully virulent virus. Vaccination with the virulent virus was always fatal.

Jansen (1942) reported infection of a jackdaw with canary pox virus. Fowls, pigeons, and canaries were susceptible to the virus. Immunity against the jackdaw strain was not produced by vaccination with fowl pox virus or pigeon pox virus but was demonstrated with canary virus.

Jactot et al. (1956) isolated canary pox virus and showed that it was pathogenic for the sparrow, fowl, and pigeon, but in the latter two species the disease was local and recovery was spontaneous. The virus did not immunize pigeons against pigeon pox, and pigeons immunized against pigeon pox were susceptible to the virus. Fowl pox and pigeon pox immune fowl were susceptible to the virus.

Pox in the mourning dove has been reported by Kossack and Hanson (1954) and by Locke et al. (1960).

Pathology. Detailed descriptions of the pathological processes in fowl pox are reported by Goodpasture (1928) and Hutyra et al. (1938).

Fowl pox is characterized by the appearance of cutaneous eruptions or wart-like nodules on the unfeathered parts of fowl and diphtheritic membranes of the mouth. The lesions are observed particularly about the head region, but they may also appear on the legs and feet, around the cloacal aperture, and under the wings. The characteristic lesion of the cutaneous form is a local epithelial hyperplasia involving both epidermis and underlying feather follicles with the formation of nodules. The cutaneous nodules may be very numerous or few in number, and they do not necessarily erupt at the same time. At first, the nodules appear as small, whitish foci which rapidly increase in size

and become yellowish in color as they develop. In some instances, closely adjoining lesions may coalesce, and the larger developing lesions are rough, and gray or dark brown in color. After about two weeks of development, sometimes sooner, the lesions may show areas of inflammation at their base and become hemorrhagic. The lesion then undergoes a process of desiccation and scab formation which may last for another week or possibly two weeks. In uncomplicated cases the process ends with desquamation of the degenerated parts of the epithelial layer. If the desiccated scab is removed in the meantime, a moist, sero-purulent exudate is found underneath, covering a bleeding, granulating surface. When the scab drops off, a smooth scar may be present, although in milder lesions there may be no noticeable evidence of scar tissue. The specific process is often modified by the invasion of bacteria which propagate in the degenerated epithelium and may reach the deeper layers of the mucous membrane where they cause suppurative or necrotic processes with the formation of fibrinous deposits.

The eruptions on the mucous membranes are white, opaque, slightly elevated nodules. These processes rapidly increase in size, often coalescing to become a yellowish, cheesy, necrotic material with the appearance of a pseudomembrane. When these pseudomembranes are removed they leave bleeding erosions. The invasion by contaminating bacteria aggravates the diphtheritic form of the disease. The inflammatory process may extend from the mouth region into the sinuses, particularly the infraorbital sinuses, resulting in a tumorlike swelling, and may extend into the pharynx, resulting in respiratory disturbances.

Stafseth (1931) reported that naturally infected pigeons showed typical pox lesions on various parts of the body. Most of the scabs were found on the feet, legs, and near the base of the beak. A few pigeons showed cankers in the mouth. Hutyra et al. (1938) reported that in pi-

FIG. 26.1—Epithelial changes in fowl pox. ×460. (Biester, Iowa State University.)

geons the pox lesions develop chiefly on the borders of the eyelids, the angles of the mouth, and on the feet.

The characteristics of the canary disease of Kikuth and Gollub (1932) show little resemblance to fowl pox, but Burnet (1933a) and Burnet and Lush (1936) presented evidence that the virus was a member of the bird pox group and classified it as canary pox. Intramuscular injection of the virus produced an inflammatory necrotic local lesion and a generalized viremia. The postmortem appearance resembled that of a subacute bacterial cellulitis. Hemorrhages under the serous membranes, edema of the lungs, and pericarditis were observed. The difference between the pathological manifestations of fowl pox infection and Kikuth's canary pox infection was the capacity of the canary pox virus to multiply within the cytoplasm of the cells derived from all three primary germinal layers. Mononuclear cells were infected and spread the infection in many respects comparable to the spread of a pyogenic bacterial infection.

Durant and McDougle (1938) observed that in canaries the external pox lesions affected the entire body. Scab formation was not as distinct as in chickens, although there was a tendency toward this condition in the advanced stages of the disease. Cheesy exudates were found in the commissures of the mouth and the entrance to the larynx.

Canary pox, as reported by Coulston and Manwell (1941), may be manifested by lesions about the margin of the epithelium of the eye and elsewhere on the head, and by lesions of the toes and legs.

The significant feature of the histological picture of fowl pox is the presence of intracytoplasmic inclusion bodies in the affected epithelial cells (Fig. 26.1). Goodpasture (1928) states that only squamous epithelium of the skin and mucous membranes seems to be susceptible to the virus and that the basal layer of the epithelium shows little change from the normal. The early stages of the inclusion bodies appear peripheral to the basal layer. The cells in which they are present are enlarged, and the cytoplasm appears edematous. The material constituting the inclusion body appears first about the periphery of one or more vacuoles situated at the proximal pole of the cell. The inclusion body increases rapidly in size, in some cases becoming as large as the original cell.

Nearer the surface, the altered cells become larger, and there is evidence of change in position of the inclusion bodies so that they come to lie more frequently at the distal pole of the cell. As the surface of the lesion is approached, the cells show evidence of disintegration, and the inclusion body may become more or less isolated in the cytoplasm. The increasing size of the inclusion body is accompanied by the ultimate destruction of the nucleus and the death of the cell. Extruded nuclear particles may appear in the cytoplasm. On the surface of the lesion there are great alterations of the cells from desiccation.

Inclusion bodies in the affected cells of cutaneous eruptions of fowl pox were first discovered by Rivolta (1869). Bollinger (1873), through histological studies, differentiated the lesions of fowl pox and variola and classified fowl pox among the tumors as epithelioma contagiosum. This term unfortunately exists today but is an erroneous terminology, although the gross lesions of the disease do have some macroscopic similarity to epitheliomata. Guarnieri (1892) demonstrated certain characteristic intracellular bodies in the lesions of variola differing from those of fowl pox. These studies of Bollinger and Guarnieri presented convincing pathological differentiations between the two diseases as distinct entities.

Borrel (1904) discovered, in smear preparations from the cutaneous lesions of fowl pox, myriads of minute coccuslike structures apparently small enough to pass through the pores of a Berkefeld filter. This discovery suggested that these bodies might be the etiological agent.

Burnet (1906) confirmed Borrel's observations and showed that these bodies were derived from the same cell in which inclusion bodies could be demonstrated in stained preparations. Goodpasture (1928) reported that these bodies were about 0.25μ in diameter.

The experiments of Woodruff and Goodpasture (1929) on inclusion bodies of fowl pox presented conclusive evidence as to the etiological agent. These authors discovered that a 1 per cent solution of trypsin in 0.2 per cent sodium bicarbonate would dispel completely the cellular material of a fowl pox lesion in about 30 minutes, leaving the inclusion bodies free and separable from the tissue debris. The inclusion bodies were small, oval, occasionally bean shaped or irregular, and varied from 2 or 3μ to perhaps 50μ. In their internal structure, they ranged from discrete granules to hyalinelike, homogeneous bodies. Inclusion bodies had an elastic, semipermeable membrane of lipoproteid composition and responded to osmotic influences. Inclusion bodies could be washed free of trypsin by centrifugation and resuspending the bodies in saline. Woodruff and Goodpasture showed that a single inclusion body inoculated into the skin of a susceptible chicken produced a typical fowl pox lesion containing the characteristic inclusions.

Woodruff and Goodpasture (1930) showed that an inclusion body may contain as many as 20,000 elementary bodies, each of which was capable of inciting the disease.

Studies by Woodruff and Goodpasture (1931) of chorio-allantoic membranes of chicken eggs infected with fowl pox virus showed a marked susceptibility of ectodermal cells to infection, although entodermal cells could be infected. Inclusion bodies were usually less numerous and hyperplasia was less marked in entodermal cells than in ectodermal cells. Further indication that entoderm was less susceptible than ectoderm was seen in the fact that entodermal derivatives of the adult hen were rarely infected.

Brandly (1941) concluded from studies of infected chorio-allantoic membranes that "both fowl- and pigeon-pox viruses produced microscopic retrograde changes in all three germ layers of the chorio-allantois. Fowl-pox strains produced more severe alterations in the ectodermal and entodermal layers, while with the pigeon virus the reactions were more marked in the mesoderm (cellular infiltration and

edema). The development of cytoplasmic inclusions (Bollinger bodies) produced by fowl strains appeared to be associated with necrosis of the limiting cellular layers. As a rule, these inclusions in the ectoderm and entoderm were larger and more readily demonstrated than those occurring in pigeon-virus lesions Entodermal involvement by pigeon virus was invariably slight."

Groupé et al. (1946) showed by electron microscopy that the elementary bodies of fowl pox closely resemble those of canary pox and have many characteristics in common with the elementary bodies of vaccinia. The bodies were approximately rectangular, occurred singly, in pairs, and in short chains, and were most frequently attached to one another at the corners.

Groupé and Rake (1947) stated that "Although the elementary bodies of fowl pox are somewhat larger than those of vaccinia, the similarities between the two are striking. The particles of both appear to be approximately rectangular in shape and possess large central moundlike elevations. ... That classic examples of both avian and mammalian strains should so closely resemble one another morphologically adds still another link to the chain of evidence that has bound the viruses of the pox group together. The presence of forms suggesting unequal division of elementary bodies, together with the characteristics mentioned above, supports the suggestion of Green et al. (1942) that the pox viruses have morphological characteristics that approach those of the bacteria rather than those of the plant viruses." (Fig. 26.2.)

Burnet (1933a) observed well-marked cytoplasmic inclusion bodies in the epithelial cells of skin overlying lesions produced by Kikuth's canary pox virus. There was a considerable resemblance of these inclusions to the Bollinger bodies of fowl pox. On the chorio-allantoic membrane the virus produced massive inclusions in
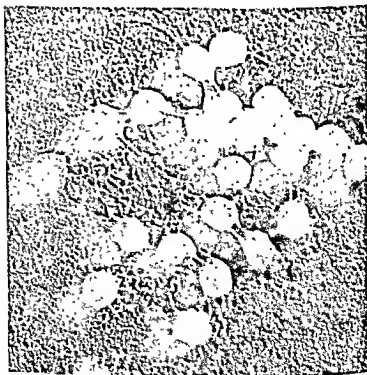
FIG. 26.2 — Fowl pox virus shadowed with gold. Electron micrograph. 2.3 x 12,400X. (Groupé and Rake, 1947.)

the proliferating ectoderm, but changes in the mesodermal and entodermal layers appeared to be purely secondary and no inclusion bodies were observed.

Durant and McDougle (1938) reported the findings of their histological studies of canary pox as follows: "Microscopic studies of the stained sections from canary pox lesions in canaries, chickens and turkeys showed distinct differential characteristics. In the sections from canaries numerous typical virus inclusion bodies are present with very few if any polymorphonuclear eosinophils with rods. In the section from chicken lesions there are no virus inclusion bodies evident and only a few polymorphonuclear eosinophils with rods. In the case of turkeys the sections showed a marked infiltration of polymorphonuclear eosinophils with rods with small bodies resembling virus bodies which are usually seen in the canary pox or fowl pox lesions. These are observed only in the deeper tissues. . . ."

Coulston and Manwell (1941) observed typical cellular inclusions in canary pox.

A variant strain of canary pox virus studied by Siegel and Leader (1957) produced cutaneous papular eruptions when introduced into the scarified, defeathered areas of the skin of the chicken, turkey, and canary. In the chicken and turkey the lesions were characterized by marked inflammation in the corium and subcutaneous connective tissue with the epithelium remaining essentially normal. In the canary there was massive proliferation of epithelium with numerous intracytoplasmic inclusions as well as severe subcutaneous lesions. The gross lesions in the chicken and turkey were mild and transitory, but in the canary they became confluent and formed thick scabs.

An excellent review of the literature on canary pox is presented by Beaudette (1953).

Diagnosis. The presence of cutaneous lesions typical of fowl pox in chickens usually warrants a diagnosis of the disease. A diagnosis is not so readily made when mouth cankers or coryzalike lesions and symptoms are seen. Several diagnostic tests may be employed, viz., infectivity tests, protection tests, microscopic examination of lesions, and serological tests as described by Brandly and Dunlap (1938).

With infectivity tests it may be necessary to utilize heterologous as well as homologous hosts for an accurate diagnosis of the type of pox virus present in the bird. Fowl pox virus may be demonstrated readily by the application of a suspension of the material to the skin of susceptible chickens by scarification of the comb or by the "stick and "feather follicle" methods described later. If the virus is present the chicken will develop typical cutaneous lesions in from 5 to 7 days. In atypical cases microscopic examinations should be made of scrapings from the base of the lesions for detection of Bollinger bodies.

Protection or immunity tests with fowl pox immune and susceptible chickens may be used simultaneously with infectivity tests and microscopic tests. The original fowl pox suspected birds which recover from the infection may be tested for immunity to a known fowl pox virus.

Microscopic examinations of smears and histological preparations of suspected lesion material are of diagnostic value. Smears may be prepared according to the method of Goodpasture (1928): "If the surface of such a lesion be slightly scraped and the scrapings moistened with water and pressed under a cover glass, the virus bodies may be easily recognized under the microscope. . . . In smears stained with Loeffler's flagella stain, or better with carbol-anilin-fuchsin for 1 minute, after mordanting an equal period with ¼ per cent potassium permanganate (Goodpasture), they are readily demonstrable. They are round or slightly oval, sometimes arranged in short chains or in diplococcal and biscuit forms. They are colored a distinct pink by the latter method, and are Gram-negative. These bodies may be studied equally well in darkfield preparations. They measure about 0.25μ in diameter, and have the appearance gener-

ally of a minute, nonmotile microorganism."

Brandly and Dunlap (1938) utilized the method of Morosow (1926) for preparing and staining direct smears from suspected lesion material. These authors have successfully used this method of examination to facilitate rapid diagnosis of suspicious field cases.

Angstrom (1951) reported that Bollinger bodies are readily demonstrable in fresh, unstained preparations from recently formed lesions.

Sevoian (1960) described a rapid, histological method using a solution which fixes and dehydrates tissues simultaneously. Using hematoxylin and eosin stains, intracytoplasmic inclusions for avian pox were demonstrated readily within 3 hours.

Stained histological preparations should reveal the presence of Bollinger bodies and the typical microscopic lesions observed with fowl pox.

Serological tests of the suspect virus with known fowl pox antiserum may be conducted with living hosts and also with embryonating chicken eggs as described by Burnet (1936b) and Burnet and Lush (1936) for neutralization tests. Burnet and Lush (1936) produced fowl pox antiserum by inoculation of susceptible birds on the scarified comb and intramuscular injection of an emulsion of fowl pox infected chorio-allantoic membrane material. A course of three more intramuscular injections of infected membranes was begun 3 weeks after the initial inoculation. Blood was collected from the birds 10 days after the last inoculation.

Dalling et al. (1929) were successful in producing fowl pox antiserum by immunizing susceptible chickens through application of the virus to the scarified comb. After "takes" were no longer produced by subsequent application of the virus to the comb, a course of four to six increasing doses of active virus was injected intramuscularly. The protective value of the antiserum could be estimated by two methods — scarification and intravenous injection. With the scarification method,

equal amounts of the test-dilution virus and antiserum were admixed, incubated at room temperature for 1 hour, and then applied to the scarified comb. It was possible to test as many as twelve samples on the comb of one bird. With the intravenous method, varying amounts of antiserum were injected intramuscularly, and on the following day each bird received the test-dilution of virus intravenously.

Neutralization tests utilizing embryonating chicken eggs would necessitate the recovery of the virus in a bacteria-free inoculum. Techniques are discussed under the section Cultivation of avian pox viruses. Isolation and propagation of the virus in eggs with the appearance of typical lesions on the chorio-allantoic membrane would offer presumptive evidence of the identity of the virus. Identification of the virus requires neutralization tests using the embryo technique, protection or immunity tests, or serological tests.

Ledingham (1931) demonstrated agglutination of the elementary bodies of fowl pox virus with sera in dilutions as high as 1:160 from recovered and hyperimmunized chickens. Agglutination did not occur with normal sera. Quantitative experiments (Ledingham, 1932) showed that the agglutinin response was slow after inoculation with the virus. Sera from hyperimmunized birds showed titers as high as 1:300.

The agar gel precipitation test can be used for the diagnosis of fowl pox and its differentiation from certain other respiratory diseases of chickens according to Jordan and Chubb (1962) and Woernle (1963). The test is relatively simple to perform and can be completed within a few days in comparison to the longer time and more technical aspects of using chicken embryo methods.

Cultivation of avian pox viruses. Fowl pox virus like other viruses can be cultivated only in living cells. These requisites may be supplied by the host, avian embryos, and tissue culture.

Cultivation of fowl pox virus in the host may be accomplished by application

of the virus to the scarified comb of a susceptible chicken and collection of the scabs. Brandly and Bushnell (1932) reported that lesion material harvested the tenth and eleventh days after inoculation was the most virulent and produced the most extensive cutaneous lesions. The scabs should be desiccated, powdered, and stored under refrigeration until used for the next passage. Pigeon pox virus may be propagated, according to Graham and Brandly (1940), by the application of a freshly prepared 1 per cent aqueous solution of powdered pigeon pox skin lesion material to a defeathered area of the ventral surface of the breast of pigeons. Inoculated pigeons were killed when moribund, usually about the sixteenth day, and the affected skin and scab removed. This material was desiccated, cut into small pieces, ground to a fine powder, and refrigerated until used for the next passage.

The technique of avian embryo culture of viruses requires bacteria-free inoculum. Woodruff and Goodpasture (1931) successfully cultivated the virus of fowl pox on the chorio-allantoic membrane of chicken eggs. Woodruff and Goodpasture (1931) and Beaudette and Hudson (1938) described certain aseptic techniques for collection of bacteria-free, virus-infected materials from birds. Penicillin and streptomycin, however, can be used to render such material bacteria-free. Variable amounts of these antibiotics have been employed but, as a general rule, 1,000 to 10,000 Oxford *units* of penicillin and 1 to 10 mg. of streptomycin per milliliter of suspended material are satisfactory. No brega and Reis (1949) used 5,000 Oxford units of penicillin to free suspensions of pigeon pox virus-infected skin of bacterial contamination.

The Bierbaum and Gaede (1935) method of intracerebral inoculation passage through pigeons and/or chickens of pigeon pox lesion material for obtaining bacteria-free virus inoculum has been utilized successfully by Brandly and Dunlap (1938), and Brandly (1941). This method consists of introducing the material intra-

cerebrally, and after 8 to 12 days incubation the bird is sacrificed and the brain removed aseptically. Brandly (1941) reported that in two instances a pure pox virus was obtained from the first passage, and rarely were three or more intracerebral passages necessary to free the virus from bacteria.

Avian embryo culture offers an economical and convenient means for the pursuit of many fundamental virus investigations as well as the source of materials rich in virus for the production of vaccines free of bacteria. Consideration must be given to the species of embryos, as certain viruses fail to propagate in embryonating eggs of all domestic fowl. The temperature of incubation and the age of the embryos also influence the action of the virus, as well as the route of inoculation (Cox, 1952; Cunningham, 1963).

There are two general methods, with modifications, for preparing eggs for inoculation of the chorio-allantoic membrane with virus (Cunningham, 1963). The Brandly (1935, 1936, 1941) method consists of introducing the inoculum between the inner shell membrane and the chorio-allantoic membrane.

The Burnet (1936a) method consists of the production of an artificial air cell on the side of the egg where the inoculum is deposited on the chorio-allantoic membrane.

The amount of inoculum depends upon the particular problem under consideration. Inoculum of 0.05 cc. or more has been used by various investigators.

A technique which permits, in a single injection, a combination inoculation of the chorio-allantoic membrane, allantoic chamber, and yolk chamber has been described by Gorham (1957).

Woodruff and Goodpasture (1931) used 10- to 15-day-old embryos for their investigations of fowl pox virus. They were, however, successful in cultivating the virus on the chorio-allantoic membrane of 6-day-old, and, in one instance, 4-day-old embryos. Slight abrasion of the skin of the embryos resulted in successful growth of

the virus, but the trauma resulting from this method was so great and the mortality so high that this method was abandoned.

Burnet (1936a) reported that for the study of inclusions 10-day-old embryos are best suited, while for virus production 11- or 12-day-old embryos are preferred. Brandly (1936, 1937, 1941) used 10- to 14-day-old embryos, but 12-day-old embryos were preferred for critical observations of the action of the virus.

Brandly (1937) studied the susceptibility of duck, guinea fowl, and turkey eggs to fowl pox virus as compared to chicken eggs. The eggs were of various ages from 10 to 18 days with the control chicken eggs 12 days old. Infection was obtained in all species of eggs employed but a ten- to thirty-fold greater end-point concentration of the virus was necessary to initiate infection in duck eggs. This was interpreted as a lower degree of susceptibility of duck-egg membranes to infection with fowl pox virus. An appreciable increase in the resistance of duck eggs was noted after the fifteenth day of incubation. Infection was not noted in duck eggs inoculated on the eighteenth day, while slightly more than half of the eggs inoculated on the sixteenth day showed evidences of infection. Turkey eggs of the same age as the duck eggs and inoculated with the same virus preparation showed infection of 80 per cent of the eggs. With chicken eggs it was found in some instances that infection was obtained in 10- and 12-day-old eggs with virus concentrations approximately 10 to 30 times smaller than was required to infect 14-day-old eggs. A tendency to metastasis of pock lesions was noted on the membrane of the 10- and 12-day-old eggs when dilute suspensions of virus were used. Large confluent lesions confined to the large pole of the egg were found in about equal numbers of the eggs of the different ages which received concentrated virus. Differences in the survival time of the embryos in eggs of various ages that developed pox infection did not appear consistent or significant. The infec-

tive concentration of fowl pox virus was not materially influenced by adsorption when powdered Pyrex glass and quartz sand were used as abrasives for grinding infected egg membranes. Repeated egg passages through 20 successive series of eggs did not change the virus insofar as the appearance of skin lesions in vaccinated birds was concerned. It was recommended that all harvested material be rapidly dehydrated at a low temperature if the material was to be held an appreciable length of time.

Brandly and Dunlap (1939) reported that passage of one strain of fowl pox virus through 68 series of eggs did not influence the virulence for chickens or for the chorio-allantoic membrane. The membranes of 12-day-old eggs were found to be more susceptible to fowl and pigeon pox virus than was the skin of chickens 6 to 12 weeks old.

Brandly (1941) conducted extensive experiments on the propagation of fowl and pigeon pox viruses in chicken eggs and the utilization of these viruses in immunization studies. Inoculated 12-day-old embryos were incubated for 4 to 5 days and the chorio-allantoic membranes were collected. Gross lesions were visible as early as 48 hours after inoculation. The pigeon pox viruses had the tendency to localize over the area inoculated. The fowl pox viruses tended to metastasize rapidly. According to Brandly, "The nature of the lesions induced in developing chicken eggs by fowl pox and pigeon pox viruses differed considerably among the strains studied. Grossly, the pigeon virus lesions were typically pale yellow to white, with a nacre or pearly tint, whereas the fowl virus infected membranes were usually reddish gray and quite heavily congested. Individual pocks . . . were globular in form in the case of pigeon virus, compared with the somewhat thinner and relatively flat fowl pocks." (Fig. 26.3.)

Thorning et al. (1943a) presented evidence of fowl pox virus in the embryo proper and yolk as well as in the chorio-allantoic membrane. The greatest concen-
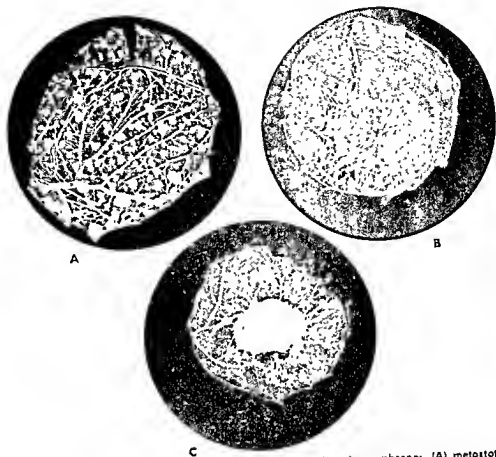
FIG. 26.3 — Fowl and pigeon pox lesions on chorio-allantoic membrane: (A) metastatic fowl pox lesions, (B) diffuse fowl pox lesions, (C) focal pigeon pox lesion. (Brandly, Ill. Agr. Exper. Sta., Bul. 478.)

tration of the virus was in the chorio-allantoic membrane, a lower concentration in the yolk, and a still lower concentration in the embryo proper. They (1945b) stated that "Available evidence does not indicate that multiplication of fowl pox virus occurs in various parts of the embryo exclusive of the chorioallantois, and until further evidence is presented, it appears that the virus content, as well as its immunogenic activity, is related to the virus content of the chorioallantois."

In Beaudette and Hudson's (1938) report on the cultivation of pigeon pox virus on the chorio-allantoic membrane of chicken eggs, it is stated that the lesions did not differ in appearance from those of fowl pox virus. The most extensive involvement was seen when the inoculum was rich in virus and the eggs were incubated more than 5 days following inoculation. Membranes removed from eggs near the hatching date contained little or no virus. One trial with 8-day-old pigeon eggs showed by inoculation of chickens with the harvested membrane that the virus was active, but there was no evidence of the growth of the virus on the membrane since lesions were not produced. Fowl pox virus was apparently lethal to 12-day-old Muscovy duck embryos whereas it had but little effect or lethal action on chicken embryos. Duck embryos were red, but the membranes were not thickened

According to von Reischauer (1906), the virus resists dry heat for 15 to 30 minutes at 80° C. and moist heat for 5 minutes at 100° C. The virus is inactivated in 5 minutes by 1 per cent potassium hydroxide, 1 per cent acetic acid, and bichloride of mercury 1:1,000. Loewenthal (1906) exposed the virus to radium and found it active after 5¼ hours. An emulsion of scabs was active after 1¼ hours in 1 per cent phenol but not in 2 and 2½ per cent phenol according to Marx and Sticker (1903), Graham and Barger (1936) found that 1 per cent aqueous suspension of fowl pox virus on sterile cotton squares, on the feet and down of day-old chicks, upon being subjected to routine incubator fumigation, survived 30 minutes, often 45 and 50 minutes, but was consistently noninfective after 90 minutes. The infectivity of the virus suspension fumigated for 30 and 45 minutes was not appreciably altered as demonstrated by infectivity tests with susceptible chicks.

Graham and Brandly (1940) reported that 1 per cent suspensions of virus containing 0.025 to 0.5 per cent formalin, 0.5 per cent phenol, 2 per cent saponin, and 0.5 per cent tricresol were completely inactivated when stored for 48 hours at icebox temperatures. Coulston and Manwell (1931) found that canary pox virus in the dried form was virulent for 7 months but not for 11 months. Beaudette (1941) reported that a dry scab removed from a wild turkey and stored in an electric refrigerator contained active virus approximately 8 years later.

McCulloch (1915) enumerates several factors to be considered for an assessment of the virucidal properties of physical and chemical agents. The author reported that 95 per cent and 75 per cent solutions of ethyl alcohol inactivated the virus in less than 10 minutes, 50 per cent ethyl alcohol inactivated the virus in 30 minutes but not in 10 minutes, and 25 per cent ethyl alcohol was without effect. These tests were made at 20° C. with 1,000 infective doses, and the broth in which the virus was tested was at pH 7. The virus was found to be inactivated by 50 parts of available chlorine per million when the material was suspended in F.D.A.[1] broth. The virus was able to withstand a 20-minute exposure to 3 per cent formaldehyde solution at 20° C., although the incubation period in chickens inoculated with the treated virus was prolonged. A commercial solution of hexylresorcinol (1:1,000) inactivated the virus when diluted 1:4 but not when diluted 1:8. Tincture of iodine diluted 1:400 inactivated the virus, but a 1:800 dilution was without effect. A 1 per cent aqueous solution of mercurochrome was without effect, while a 2 per cent solution inactivated the virus at 20° C. at pH 7. The virus was able to withstand 3 per cent phenol for 10 minutes at 20° C. but not for 30 minutes. A 1:500 dilution of sodium hydroxide at 20° C. inactivated the virus in 10 minutes, but a 1:600 dilution was without effect. When the virus was suspended in F.D.A. broth pH 7, at 20° C., 1:1,000 crystal violet inactivated the virus in 10 minutes, 1:50 acriflavine in 10 minutes but not 5 minutes, while 1:100 acid fuchsin failed to inactivate the virus in 30 minutes. In each inoculum there were approximately 1,000 infective doses. A 1:400 dilution of liquor cresolis at 20° C. inactivated the virus in 10 minutes but not in 5 minutes, while at 20° C. a dilution of 1:5,000 was as effective as the same dilution at 40° C.

A suspension of 100,000 infective doses of the virus in F.D.A. broth was inactivated in approximately 5 minutes at 60° C., in 15 to 20 minutes at 55° C., and resisted for longer than 1 hour at 50° C.

Graham et al. (1939) reported that irradiation of aqueous suspensions of fowl pox virus with hard X-rays in dosages up to 888 r units had no effect. The virus was inactivated by ultraviolet light from a mercury vapor lamp in 2 hours at 20 cm. and attenuated when exposed for 15 to 90 minutes. The addition of methylene blue in a concentration of 1:50,000 reduced

[1] Food and Drug Administration

the time necessary for inactivation to 5 minutes, while 2½ minutes irradiation markedly attenuated the virus.

Robbins (1944) reported that neither penicillin nor patulin were effective against the virus as shown by infectivity tests on the chorio-allantoic membrane of chicken eggs.

Beaudette et al. (1948) found that fowl pox virus propagated in chicken eggs was active after being held in the dried state up to 3,598 days, while the same strain adapted to duck eggs was active up to 1,928 days. Pigeon pox virus passaged in chicken eggs and in duck eggs was active up to 3,605 and 1,099 days, respectively.

Epizoology. Fowl pox is prevalent wherever poultry is raised. The incubation period of the spontaneous disease varies from 4 to 6 days, according to Goodpasture (1928), and from 6 to 14 days, according to Delaplane (1943). While the disease may make its appearance at any time, the greatest incidence is during the fall and winter months. Under natural conditions, the disease usually makes its appearance in young stock at about the time they are housed in laying quarters. Following this it may reach serious proportions. During the fall and early winter, the cutaneous form predominates in most outbreaks, while during the winter months the diphtheritic form is usually most common. The epizoological picture is similar to that of other contagious diseases in that variations in virulence may be observed. If nothing is done to control an outbreak, it may persist in a flock throughout the winter. The course of the uncomplicated disease is usually about 3 to 4 weeks, but if complications are present the duration may be considerably longer. Recovered birds are immune to further infection.

Chickens affected with the cutaneous form of the disease are more likely subjects for recovery than chickens affected with the diphtheritic form, particularly when the lesions involve the respiratory tract, eyelids, and nasal sinuses. A flock in good physical condition usually war-

rants a favorable prognosis, while the prognosis is unfavorable if the flock is affected with other infectious or parasitic diseases or is subject to poor nutrition and management.

The mortality rate is variable. In laying flocks the egg production will be temporarily retarded. This is associated with an increasing number of emaciated chickens. The mortality in laying flocks may assume serious proportions.

Individual symptoms of fowl pox may be manifested in one of three forms or a combination of these forms, depending upon the virulence and pathogenicity of the strain of virus involved: (1) localization of typical cutaneous pox lesions on the comb, wattles, and face region (Fig 26.4); (2) localization of the infection in the mouth region with the appearance of typical diphtheritic lesions; and (3) localization of the infection in the nasal chambers with accompanying coryzalike symptoms. While pox lesions are not commonly observed on the feet, legs, and body of chickens, these lesions may be observed if chickens are reared on wire floors. Other than the typical cutaneous and diphtheritic lesions there are no characteristic lesions to be found at postmortem examination of affected chickens.

Chickens of all ages, sexes, and breeds, unless previously exposed, are equally susceptible to the virus by inoculation Under natural conditions there seem to be possible breed differences in susceptibility. Cary (1906) reported that chickens with large combs seem to be more susceptible to infection than chickens with small combs. Johnson (1927) has reported that Leghorns appear to be more susceptible to natural infection than are Barred Plymouth Rocks because of the large comb area.

The disease is not commonly seen in young chickens, although Beaudette (1929) and Johnson (1938) have observed outbreaks in battery brooded chicks. In the latter report, the chicks were 6 weeks old, and the lesions in practically all cases

FIG. 26.4 — Severe case of fowl pox. (Brunett, Cornell Vet.)

were found on the feet and legs. The absence of lesions on the combs and wattles was attributed to the lack of development of these organs at this age.

Fowl pox virus is unable to penetrate intact epithelium. Doyle and Minett (1927) were unable to induce infection by the daily application of virus to intact epithelium of the combs of chickens. Injection of virus subcutaneously, intramuscularly, intraperitoneally, and instillation in the conjunctival sac resulted in infection. Intravenous injection of the virus produced the disease in three forms: (1) generalized infection with skin or mouth lesions; (2) slowly progressive emaciation without lesions; and (3) immunity without lesions or loss of condition. From transmission experiments, the authors concluded that infection of the skin resulted from injuries sustained in picking, and that mouth lesions were the result of injuries produced by eating of grit. The intimate cohabitation of chickens in most flocks enhances the possibility of spread of the infection since most chickens in a flock present injured skin surfaces so that there is no lack of suitable portals of entry of the virus.

The possibility of "carrier" chickens serving as foci of fowl pox infection has been the subject of considerable speculation and research work. According to Doyle and Minett (1927) and Doyle (1930) several investigators have indicated that pigeon pox virus may localize in certain internal organs and may persist for a considerable length of time in these organs in recovered pigeons. Doyle (1930) stated it is possible that the frequently repeated statement that fowl pox virus acts in a similar manner is based on this work. Doyle and Minett (1927) found that fowl pox virus could be demonstrated in variable quantities in the blood of chickens throughout the course of the disease following intravenous injection or application of the virus to scarified areas of the comb and in the mouth. In no instance was the virus demonstrable in

the internal organs of recovered chickens or on the comb after complete disappearance of lesions.

According to Burnet (1906), if feathers are plucked or the skin is scarified after intravenous injection of the virus, specific lesions will develop at the site of the cutaneous injury. The author concluded that the requirements for the development of eruptive lesions are a viremia and a susceptible point for the lesion.

Beaudette (1941) stated that in a small percentage of birds (usually less than 5 per cent) secondary lesions may appear on the head as a result of viremia.

That fowl pox was transmitted by intermediary carriers has been reported by several investigators. Cary (1906) stated that "Mosquitoes — and other insects — may sometimes be the real carriers of the real virus." Kligler et al. (1929) have shown that Culex pipiens and Aedes aegypti are capable of transmitting the disease from infected chickens, as lesions developed in from 5 to 10 days after the infected mosquito was allowed to feed on a susceptible chicken. The mosquitoes were considered to be mechanical carriers of the virus as they were capable of transmission of the disease immediately after feeding on infected chickens. In one case the mosquitoes remained infectious for at least 14 days.

Kligler and Ashner (1929) studied the transmission of fowl pox by Culex pipiens and Aedes aegypti and showed that the same mosquito may produce a number of consecutive infections for at least 16 days. Infected mosquitoes were capable of transmitting the infection despite intermediate feeding on guinea pigs. The virus appeared to be localized on the proboscis of the mosquitoes, which may remain infective for 16 to 19 days. Only rarely could the disease be transmitted by inoculation of other parts of the mosquito's body. The virus behaved in the same manner as on infected pins as on the proboscis. Blanc and Caminopetros (1930) showed that infected Culex pipiens could transmit the infection for at least 58 days and from pigeon to

pigeon for at least 38 days. Matheson et al. (1932) showed that the virus could be transmitted by Aedes vexans for at least 27 days.

Stuppy (1932) presented evidence that Culex pipiens and Stegomyia fasciata were capable of transmitting fowl pox for at least 39 days. The incubation of the mosquito-transmitted infection was from 6 to 8 days. The infection was sufficient to produce immunity. It was believed that the transfer of virus was not simply mechanical and that it was possible that the mosquitoes remained infective for life. The virus was present in the body of the mosquitoes.

The incubation period of Kikuth and Gollub (1932) canary pox is about 4 days, the canaries dying from 7 to 12 days after infection. Durant and McDougle (1938) observed that canary pox apparently occurs in cycles of about 21 days in young canaries, with a mortality of about 100 per cent of all birds affected. Death occurred quite regularly from the tenth to the fourteenth day after exposure. Recovered canaries were refractory to subsequent exposure to a virulent virus. According to Coulston and Manwell (1941), canary pox was uniformly fatal to canaries after a week or 10 days when the lesions were localized other than on the toes and legs. In the latter form the disease was chronic, but it also killed the canary after a period of some weeks or months.

Prevention and control. Graham and Brandly (1940) and Beaudette (1949) present extensive reviews of the literature on immunization. Much of the early work was conducted with virus which had been attenuated, and in some cases inactivated, by physical or chemical means, or virus modified by passage through heterologous hosts. The immunogenic properties of these preparations were subject to wide variations. The ineffectiveness of these vaccines prompted exploration of the possibilities of utilizing virulent fowl pox virus obtained from cutaneous lesions. De Blieck and van Heelsbergen (1929) were probably the first to use on a large

scale the fully virulent virus for cutaneous immunization. That vaccination of chickens with fully virulent virus is an effective immunizing agent has been reported by many investigators who have also emphasized that certain potential postvaccination hazards may accompany this method of immunization. A review of the reasons for failures in immunization against fowl pox has been presented by Beaudette (1941).

Two types of vaccines are available for immunization of domestic fowl against pox: fowl pox vaccine and pigeon pox vaccine. The success of a program with these vaccines depends upon their utilization only under the conditions where indicated, and upon the potency and purity of the vaccines and their application.

Vaccines were formerly of two types: "chicken origin" or "pigeon propagated," and "chick-embryo origin" or "egg-propagated," depending upon the method of preparation. According to Hejl (1957), licensed laboratories in the United States are not permitted to use fowls in the production of poultry vaccines due to the possibility that the causative agents of other diseases may be contained in the crude material obtained from chicken and pigeon tissues.

Fowl pox vaccine. "Chick-embryo origin" or "egg-propagated" vaccine is prepared by propagation of the virus on the chorio-allantoic membrane of chicken eggs. The infected membranes are collected, desiccated, ground to a fine powder, distributed in suitable containers, and stored under refrigeration. The best type of containers are glass vials or ampoules in which the virus can be hermetically sealed *in vacuo* for maximum retention of potency of the virus. A suspension of 40 mg. of virus in 2 cc. of diluent is satisfactory for vaccination of 100 chickens according to Beaudette (1911).

Thorning *et al.* (1943a, 1943b) and Kerlin and Graham (1944a, 1944b) reported that fowl pox vaccines prepared from the entire chicken embryo possess immunogenic properties. Bryan (1949)

prepared a satisfactory vaccine consisting of 5 per cent fowl pox virus-infected, undesiccated entire embryo suspended in 50 per cent buffered glycerol at pH 7.6. When stored at 14° C., the vaccine was active for 8 months. When stored at 5° C., the vaccine was viable for 2 years.

Prier (1951) prepared a mixed Newcastle disease-fowl pox whole embryo vaccine in 50 per cent glycerol that immunized chickens against both diseases.

Brandly and Dunlap (1939) and Sabban (1954) present conclusive evidence that egg-propagated vaccines represent a distinct and desirable refinement over chicken-propagated vaccines. Zargar and Pomeroy (1950) isolated Newcastle disease virus from commercial fowl pox and laryngotracheitis vaccines. These findings emphasize the necessity for adequate precautions to exclude contamination of vaccines with extraneous viruses.

Pigeon pox vaccine. "Chick-embryo origin" or "egg-propagated" vaccine is prepared and processed as previously described for fowl pox vaccine. A suspension of at least 80 mg. of virus in 4 cc. of diluent for vaccination of 100 chickens is recommended by Beaudette (1941).

Nobrega and Reis (1949) reported that fowl pox vaccine prepared with whole embryo from eggs inoculated with pigeon pox virus by the allantoic cavity route gave results as satisfactory as those obtained by propagation of the virus on the chorio-allantoic membrane.

Vaccination. Fowl pox vaccine is used to vaccinate chickens and turkeys, and it may also be used for pheasants. Fowl pox vaccine is not to be used on pigeons, and it is never to be used on laying birds.

Pigeon pox vaccine is used to vaccinate chickens, turkeys, pigeons, and pheasants. It is used on chickens and turkeys when these birds are laying or when the flock is debilitated through the presence of other diseases or improper nutrition and management.

Fowl pox vaccines of adequate potency contain the causal agent and when improperly used are capable of producing

the disease in a severe form in chickens. When properly used, the vaccine is equivalent to a mild attack of the disease. Vaccination differs from the natural disease only in that the vaccine is applied to a small area, the extent of the lesion is less than that encountered in the natural disease, and it is given to the chicken at an age when postvaccination reactions are less likely to occur than in the natural disease.

All vaccines should be mixed away from the poultry house and precautions should be taken not to spill any of it. The vaccine should be mixed just before use and only enough prepared for one day's operation. If it is necessary to hold vaccine overnight, it should be kept frozen. After the vaccine is mixed, the hands should be washed to prevent contamination of the birds since the vaccine should be kept away from all parts of the bird except the site of vaccination. When vaccination is completed, the unused vaccine and all containers should be burned. The "stick" instruments should be boiled if they are to be kept for further use.

Since the flock will have to be examined for "takes," it is well to be consistent in the site of vaccination so that no confusion will occur in a false interpretation of the reaction to the vaccine.

Chickens may be vaccinated with fowl pox vaccine by one of two methods: the "stick" method and the "feather follicle," or brush, method, since the virus has a predilection for cutaneous epithelium and follicular cells. Chickens may be vaccinated successfully with pigeon pox vaccine by the "feather follicle" method only, since pigeon pox virus has a particular affinity for follicular cells.

In the "stick" method the vaccine is introduced into the cutaneous epithelium by sticking the skin with a sharp-pointed instrument that has been moistened with the vaccine. The sites of application may be the skin of the leg, the under surface of the web of the wing, or the breast. The under surface of the web of the wing is probably the most convenient area. In baby chicks the vaccine is introduced into the skin of the flank region. The vaccinating instrument may be of any type that will insure adherence of the virus for its introduction into the skin. Most manufacturers supply an instrument with the vaccine.

When the "stick" method is used, the vaccine should be in a container with a mouth wide enough to allow entrance of the instrument so that the points can be moistened easily with the vaccine. As an added precaution to prevent spilling of the vaccine, a small piece of wood, 2 inches by 4 inches by 6 inches long, with holes large enough to receive the container of vaccine, should be prepared. The instrument should be moistened with vaccine before each application.

With the "feather follicle" method the vaccine is applied to defeathered follicles with a brush. The brush should be stiff enough to withstand repeated usage without deterioration, and the bristles should be of a suitable length and number. Most manufacturers supply a brush with the vaccine. When vaccine is applied, only three or four follicles should be infected, as the reaction of the vaccine increases in proportion to the number of follicles infected. Usually, the most convenient vaccination area is the anterior or lateral aspect of one leg about midway between the hock joint and the femoro-tibial articulation of the leg. The bristles of the brush should be moistened with vaccine before each application and directed into the open follicles for proper deposition of the vaccine.

When pigeon pox vaccine is used, the area should be considerably larger than that with fowl pox vaccine, since the degree of immunity produced is in direct proportion to the size of the vaccination lesion, and pigeon pox virus does not produce a systemic reaction in chickens. The feathers should be plucked from an area of about 1 by 2 inches on the leg, and the exposed follicles over the entire area infected with the vaccine by directing the brush against the openings of the follicles. This is of particular importance in

view of the affinity of pigeon pox virus for follicular cells.

The method of vaccination is a matter of personal preference. There are certain distinct advantages of the "stick" method over the "follicle" method which make the former more desirable when using fowl pox vaccine. According to Johnson (1934), some advantages of the "stick" method are as follows: uses less virus; permits more rapid vaccination; requires less help by eliminating feather plucking; results in less contamination of fowls and premises; results in less virus at "take"; standardizes vaccination procedure; results in less exposure of the "takes"; and provides a method applicable to fowls from a day old to maturity.

Reaction to fowl pox vaccine and evidence of a successful vaccination consists of a scab or "take" which may be readily detected in a week at the site of vaccination by either the "stick" or "feather follicle" method. The flock should be examined for "takes" between the sixth and tenth day following vaccination. If the vaccine was applied to the web of the wing by the "stick" method, two small scabs will be observed where the points of the vaccinating instrument were introduced into the skin and usually two small scabs on

the outer surface of the wing where the points emerged (Fig. 26.5).

With the "feather follicle" method there will be a swelling of each follicle and the formation of a scab (Fig. 26.6). These scabs will enlarge during the course of a week or 10 days and may coalesce to form a single scab covering the entire vaccination area.

A "take" following vaccination with pigeon pox vaccine is indicated by a swelling of the follicles which may be evident as early as the fifth day. Scabs are not produced in chickens immunized with this vaccine. A flock examination for "takes" should be made at about 10 to 12 days following vaccination. "Takes" with both fowl and pigeon pox vaccines subside and disappear about the third week following vaccination. The scabs dry and drop off the birds immunized with fowl pox vaccine.

The immunity developed following vaccination with fowl pox vaccines endures throughout the life of the chicken. With pigeon pox vaccine the duration of the immunity is not as long nor as well established as that from fowl pox vaccine (Seegar and Price, 1956).

Vaccination provides no protection during the first two or three weeks, and birds

FIG. 26.5 — Web of wing of chicken. Six-day "takes," fowl pox vaccine, "stick" method. (Brunett, Cornell Vet.)
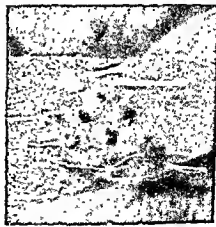
FIG. 26.6 — Leg of chicken. Six-day "takes," fowl pox vaccine, "feather follicle" method. (Brunett, Cornell Vet.)

may become infected with the natural disease. Maximum immunity is usually attained by the end of the fourth week. Vaccination is only a prophylactic measure and not a treatment. If vaccination is to be done when the disease has just made its appearance, all visibly affected birds should be removed from the flock and isolated to prevent spread of the infection from this source. Medicinal treatment of chickens against fowl pox is of no value.

When properly applied, a vaccine of adequate potency should produce "takes" in all vaccinated birds. Failure to obtain "takes" may be the result of the application of a vaccine of inadequate potency (one used after expiration date or subjected to deleterious influences), improper application of the vaccine, or use on immune birds In the event that "takes" are not obtained (except in birds previously immunized or recovered), the flock should be revaccinated at once.

The age of the bird receiving fowl pox vaccine is important. In certain areas day-old chicks are vaccinated, but the severe systemic reactions and high mortality rate following vaccination do not make birds of this age satisfactory subjects The preferable age is from the sixth to the twelfth week according to Delaplane (1943) and from the eighth to the tenth week according to Beaudette (1941). As as rule the chickens should be at least 1 month old. Chickens should not be vaccinated within 1 month and preferably 2 months before production is expected to start in order to allow ample time for them to recover from the effects of the vaccination. The upper limit for light breeds would be from 3 to 3½ months and for heavy breeds from 4 to 4½ months.

With pigeon pox vaccine the upper age limit for vaccination is not important since this vaccine does not produce a systemic reaction in chickens. In young chickens, however, the age limit depends upon the feathering of the bird since the vaccine is applied only by the "feather follicle" method. Generally, chickens should be at least 6 weeks old, and it is preferable to

have all birds in the flock well feathered before vaccination.

When pigeon pox vaccine is applied to pigeons, only four or five follicles need to be infected, since this virus is as pathogenic for pigeons as fowl pox virus is for chickens. Follicles of either the breast or leg of the pigeon may be inoculated. The bristles of the vaccinating brush should be directed against the openings of the follicles to insure the deposition of the vaccine in the follicles. Pigeons should be vaccinated at about 4 to 6 weeks of age. Vaccinated pigeons should be segregated from nonvaccinated pigeons to prevent spread of the infection.

Goldhaft (1956) has described a method of spray application of pigeon pox vaccine to chickens. The degree and duration of immunity was, in general, directly related to the number and area of follicles infected by the vaccine. The vaccine was used successfully with adult chickens and day old chicks.

Prophylactic vaccination. Prophylactic immunization of chickens against fowl pox consists of vaccinating susceptible chickens with fowl pox vaccine prior to the time when the disease is likely to appear. Vaccination is usually done during the spring and summer months in those areas where the disease appears during the fall and winter months. In tropical climates, where the disease may make its appearance throughout the year, vaccination may be done at any time when warranted without regard to seasonal periods.

Vaccination is indicated in three types of flocks which present themselves in the problem of prophylaxis against fowl pox:

1. Vaccination is indicated as a routine prophylactic measure in a flock that has been infected the previous year and when the owner wishes to prevent such an occurrence in the new susceptible population. All young stock, produced on the premises or introduced from other sources since the outbreak, should receive fowl pox vaccine. When several lots of birds are raised during the year, each lot should be vaccinated at the appropriate age. Vac

cinated birds should be maintained under strict isolation as they are *a source of infection* for the nonvaccinated birds. If vaccination with fowl pox vaccine is delayed beyond a reasonable limit to expect the establishment of immunity prior to housing of the pullets, then pigeon pox vaccine should be used. This practice, however, should not be necessary.

2. If fowl pox was present the previous year but pigeon pox vaccine was used to check the spread of the disease at that time, vaccination is desirable. Since pigeon pox vaccine does not induce a durable immunity in chickens, the old birds should be revaccinated with fowl pox vaccine.

3. *In certain congested poultry districts* where fowl pox is prevalent, the owner should immunize the flock through the application of fowl pox vaccine to protect the flock against infection from the neighboring flocks. Delay under these circumstances may result in the infection being established at a time unfavorable for vaccination.

Prophylactic vaccination of pigeons with pigeon pox vaccine is indicated if the infection was present on the premises the previous year and if the loft is in a congested pigeon district.

Vaccination of canaries against pox with canary pox virus has not been successful according to Burnet (1933a) and Durant and McDougle (1938). The latter authors observed that canaries recovered from the natural disease were refractory to further infection. Coulston and Manwell (1941) reported that canaries which had been vaccinated with the virus attenuated through storage did develop an immunity which protected them to some degree, but not completely, against exposure to fully virulent virus. As a therapeutic measure, 1 to 3 per cent mercurochrome in 70 per cent alcohol, to which a trace of acetone was added, was applied once or twice daily to the infected pox areas. The duration of the treatment varied with the severity of the disease. The authors concluded that "This method of treatment is successful in nearly all cases, unless they are very advanced. Recovered birds exhibit a strong immunity to reinfection, particularly if they have been infected to begin with by a virulent virus."

Beaudette (1949) reported that favorable results had been obtained by vaccination of young canaries by the wing "stick" method using a suspension of the most advanced passage of an egg-propagated strain of canary pox virus. Beaudette (1953) has compiled an excellent review of the literature on modification of canary pox virus and vaccination of canaries.

REFERENCES

Angstrom, C. I.: 1951. Poultry disease diagnosis in the laboratory. Proc. 87th Ann. Meet. Am. Vet. Med. Assn., p. 255.

Antonioli, D., and Romani, A.: 1940 Contribución al estudio del epitelioma contagioso del canario. *Revista de Med. Vet.* 22:326. Cited by Brunett (1945).

Barger, E. H., and Card, L. E.: 1943. Diseases and Parasites of Poultry. Third Ed Lea and Febiger, Philadelphia. P. 150.

Beach, J. R.: 1939 Report of committee on poultry diseases. Jour. Am. Vet. Med. Assn 95.615.

Beaudette, F. R.: 1929. Some aspects of fowl pox and its control. *Jour. Am. Vet. Med. Assn.* 75:563.

——: 1941 The reasons for failures in immunization against laryngotracheitis and pox. Proc. 45th Meet. U.S. Livestock Sanit. Assn., p. 127.

——: 1949. Twenty years of progress in immunization against virus diseases of birds. *Jour. Am. Vet. Med. Assn.*, 115 232.

——: 1953. The identity of canary pox and "schnappkrankheit" with notes on vaccination and modification of the virus. Proc. 57th Ann Meet. U.S. Livestock Sanit. Assn., p. 249.

—— and Hudson, C. B.: 1938. Cultivation of pigeon pox virus on the chorio-allantoic membrane. Jour. Am. Vet. Med. Assn. 93:146.

——, and Hudson, C. B.: 1941. Egg propagation of turkey pox virus. Poultry Sci. 20.79

Miller, B. R., Bivins, J. A., and Hudson, C. B.: 1942. The viability of dried viruses of avian origin. Am. Jour. Vet. Res. 3:190.

Bierbaum, K., and Gaede, H.: 1935. Die Züchtung von Geflügelpockenvirus in der Gewebekultur Arch. wiss. u prakt. Tierheilk. 69.441. Cited by Brandly and Dunlap (1958).

Blanc, G., and Caminopetros, J.: 1930. La transmission des varioles aviaires par les moustiques. Compt. Rend. Acad. Sci. France 190.954.

Bollinger, O.: 1873. Über Epitheliome contagiosum beim Haushuhn und die sogenannten Pocken des Geflügels. Arch. f. Path. Anat. u. Physiol. (Virchow) 58.349.

Borrel, A.: 1904. Sur les inclusions de l'épithélioma contagieux des oiseaux (molluscum contagiosum). Compt. Rend. Soc. de Biol. 2.612.

Brandly, C. A.: 1935. Some studies of infectious laryngotracheitis. Jour. Infect. Dis. 57.201.

———. 1936. Studies on the egg-propagated viruses of infectious laryngotracheitis and fowl-pox. Jour. Am. Vet. Med. Assn. 88.587.

———. 1937. Studies on certain filtrable viruses. I. Factors concerned with the egg propagation of fowl pox and infectious laryngotracheitis. Jour. Am. Vet. Med. Assn. 90.479.

———. 1941. Propagation of fowl- and pigeon-pox viruses in avian eggs and use of egg cultivated viruses for immunization. Ill. Agr. Exper. Sta., Bul. 478.

———, and Bushnell, L. D.: 1932. Studies of some virus diseases of fowls. Jour. Am. Vet. Med. Assn. 80.782.

———, and Dunlap, G. L.: 1938. An outbreak of pox in turkeys with notes on diagnosis and immunization. Poultry Sci. 17.511.

———, and Dunlap, G. L.: 1939. Studies on certain filtrable viruses II. Immunization against fowl pox with fowl- and pigeon-pox viruses cultivated in vivo and in vitro. Jour. Am. Vet. Med. Assn. 95-340.

Brunett, E. L.: 1934. Some observations on pox virus obtained from a turkey. Rep. N.Y. St. Vet. Coll. (1932-33) 69.

———. 1945. Fowl pox. In Diseases of Poultry Third Printing. H. E. Biester and L. DeVries. The Iowa State College Press, Ames, Iowa. P. 481.

Bryan, H. S.: 1939. Studies on certain filtrable viruses. X. Immunogenic properties of Borreliota avium suspended in buffered glycerol and in mineral oil. Am. Jour. Vet. Res. 10.284.

Burnet, E.: 1906. Contribution à l'étude de l'épithélioma contagieux des oiseaux. Ann. de l'Inst. Pasteur 20.742.

Burnet, F. M.: 1933a. A virus disease of the canary of the fowl-pox group. Jour. Path. Bact. 37.107.

———. 1933b. Unpublished. Cited by Burnet and Lush (1936).

———. 1934a. The use of the developing egg in virus research. Med. Res. Council, Special Rep. Series No. 220.

———. 1936b. Immunological studies with the virus of infectious laryngotracheitis of fowls using the developing egg technic. Jour. Exper. Med. 63.685.

———, and Lush, D.: 1936. The immunological relationship between Kikuth's canary virus and fowl-pox. Brit. Jour. Exper. Path. 17 302.

Buthala, D. A., and Mathews, J.: 1951. Use of cellular cultures of chicken embryo kidney tissue in virus studies. Cornell Vet 47 148.

Carnwarth, T.: 1908. Zur Aetiologie der Hühnerdiphtherie und Geflügelpocken. Arb. a. d. kaiserl. Gesundheitsamt 27 588. Cited by Doyle and Minett (1927).

Cary, C. A.: 1906. Chicken-pox, sore-head or contagious epithelioma in poultry. Ala. Agr. Exper. Sta., Bul. 136.

Coulston, F., and Manwell, R. D 1941. Successful chemotherapy of a virus disease of the canary. Am. Jour. Vet. Res. 2-161.

Cox, H. R.: 1952. Growth of viruses and rickettsiae in the developing chick embryo. Ann. N.Y. Acad. Sci. 55.236.

Cunningham, C H.: 1963. A Laboratory Guide in Virology. Burgess Publ. Co., Minneapolis.

Dalling, T., Mason, J. H., and Gordon, W. S.: 1929. Fowl-pox antiserum. Brit. Jour. Exper. Path. 10.16.

de Blieck, L., and van Heelsbergen, T.: 1923. Impfung gegen Diphtherie und Geflügelpocken bei Hühnern. Deutsch. tierärtzl. Wochenschr. 31.85.

Delaplane, J. P.: 1943. The differentiation of the respiratory diseases of chickens. R.I. Agr. Exper. Sta., Bul. 288.

Doyle, T. M.: 1930. Fowl pox. Rep of 11th Internat. Vet. Cong. 3.675.

———, and Minett, F. C.: 1927. Fowl pox. Jour. Comp. Path. and Therap. 40:247.

Durant, A. J., and McDougle, H. C.: 1933. Investigation of pox in canaries. Proc. 42nd Ann. Meet. U.S. Livestock Sanit. Assn., p 181.

Gallagher, B.: 1916. Epithelioma contagiosum of quail. Jour. Am. Vet. Med. Assn. 3:366.

Goldhaft, T. M.: 1956. A new method of application of pigeon pox vaccine. Jour. Am. Vet. Med. Assn. 128.596.

Goodpasture, E. W.: 1928. Virus diseases of fowls as exemplified by contagious epithelioma (fowl-pox) of chickens and pigeons. In Filterable Viruses. T. M. Rivers. Williams and Wilkins Co., Baltimore. P. 235.

Gorham, J. R.: 1957. A simple technique for the inoculation of the chorioallantoic membrane of chickens embryos. Am. Jour. Vet. Res. 18.691.

Graham, R., and Barger, E. H. 1936. Studies on incubator hygiene. IV. A note on the virucidal effect of formaldehyde on fowl pox virus. Poultry Sci. 15:48.

———, and Brandly, C. A: 1940 Immunization against pox in domestic fowl. Ill. Agr. Exper. Sta., Bul. 470.

————, Brandly, C. A., and Levine, N. D.: 1939. The effect of hard X-rays and ultra-violet light upon fowl pox virus in vitro. Cornell Vet. 29:383.

Green, R. H., Anderson, T. F., and Smadel, J. E.: 1942. Morphological structure of the virus of vaccinia. Jour. Exper. Med. 75:651.

Grosso, A. M., and Prieto, C.: 1939 Epitheliosis contagiosa de los canarios. Univ. de Buenos Aires. Instituto de Enfermedades Infecciosas. 1:No. 4. Cited by Brunett (1945).

Groupé, V., Oskay, J., and Rake, G.: 1946. Electron micrographs of the elementary bodies of fowl pox and canary pox. Proc. Soc. Exper. Biol. and Med. 63.477.

————, and Rake, G.: 1947. Studies on the morphology of the elementary bodies of fowl pox Jour. Bact. 53:449.

Guarnieri, G.: 1892. Ricerche sulla patogenesi ed etiologia dell' infezione vaccinica e variolosa. Arch. Sci. Med. 16:403. Cited by Goodpasture (1928).

Hejl, J. M.: 1957. Personal communication. Biol. Prod. Lac Soc, U.S.D.A., Washington, D.C.

Holmes, F. O.: 1948. Order Virales. In Bergey's Manual of Determinative Bacteriology. Sixth Ed. The Williams and Wilkins Co., Baltimore, Md.

Hutyra, F., Marek, J., and Manninger, R.: 1938. Special Pathology and Therapeutics of the Diseases of Domestic Animals. Vol. I, Fourth English Ed. Alexander Eger, Chicago. P. 380.

Irons, V.: 1934. Cross species transmission studies with different strains of bird pox, Am. Jour. Hyg. 20:329.

Jactot, H., Vallée, A., and Reinié, L.: 1956 Identification in France of the virus of canary pox, or virus of Kikuth, Ann. Inst. Pasteur 90-28.

Jansen, J.: 1912. Pokken bij de kauw. Tijdschr. Diergeneesk. 9:128.

Johnson, E. P.: 1933. An unusual outbreak of chicken-pox. Jour. Am. Vet. Med. Assn. 93:115.

Johnson, W. T.: 1927. Fowl-pox prevention by immunization. Jour. Am. Vet. Med. Assn. 71:750.

————. 1934 Fowl pox. Rep. 12th Internat Vet Cong 3:219

Jordan, F. T. W., and Chubb, R. C.: 1962. The agar gel diffusion technique in the diagnosis of infectious laryngo-tracheitis (I. L. T.) and its differentiation from fowl pox Res. Vet. Sci. 3:245.

Kangude, G. M., and Hanson, L. E.: 1961. "Cell agglutination" technique for quantitative titration of fowlpox virus Avian Dis. 5:139.

Kerlin, E. L., and Graham, R.: 1944a Studies on certain filtrable viruses. VI. Antigenic properties of entire embryo fowl pox vaccine. Proc. Soc. Biol. and Med. 55:225.

————, and Graham, R.: 1944b Studies on certain filtrable viruses. VII. Antigenic properties of entire embryo fowl pox vaccine Proc. Soc. Exper. Biol. and Med. 57:259.

Kikuth, W., and Golluh, H.: 1932. Versuche mit einem filtrierbaren Virus bei einer übertragbaren Kanarenvogelkrankheit. Zentralbl. Bakt. Abt. I. Orig. 125-313.

Kligler, I. J., and Ashner, M.: 1929. Transmission of fowl pox by mosquitoes: further observations. Brit. Jour. Exper. Path. 10:347.

————, Muckenfuss, R. S., and Rivers, T. M.: 1929. Transmission of fowl pox by mosquitoes. Jour. Exper. Med. 49.649.

Kossack, C. W., and Hanson, H. C: 1954. Fowlpox in the mourning dove. Jour Am. Vet. Med. Assn. 124:109.

Ledingham, J. C. G.: 1931. The aetiological importance of the elementary bodies in vaccinia and fowl-pox. Lancet 221:525.

————. 1932. The development of agglutinins for elementary bodies in the course of experimental vaccinia and fowl-pox. Jour. Path. Bact 35:140.

Locke, L. N., Herman, C. M., and King, Jr., E. S.: 1960. Case report—pox in the mourning dove in Maryland. Avian Dis. 4.198.

Loewenthal, W.: 1906. Untersuchungen über die sog. Taubenpocke (Epitheloma contagiosum) Deutsch. Med. Wochenschr 32:628.

McCulloch, C.: 1915. Disinfection and Sterilization Lea and Febiger, Philadelphia.

McGaughey, C. A., and Burnet, F. M.: 1945. Avian pox in wild sparrows. Jour. Comp. Path. and Therap. 55-201.

Marx, E., and Sticker A.: 1902. Untersuchungen über das Epitheliома contagiosum des Geflügels. Deutsch. Med. Wochenschr. 28.893. Cited by Goodpasture (1928).

————, and Sticker A.: 1903. Weitere Untersuchungen über Mitigation des Epithelioma contagiosum des Geflügels Deutsch Med Wochenschr. 29 79. Cited by Goodpasture (1928) Burnet (1906).

Matheson, R., Brunett, E. L., and Brody, A. L.: 1932. The transmission of fowl pox by mosquitoes, preliminary report. Rep N.Y. St. Vet Coll. (1930–31):177.

Morosow, M. A.: 1926. Die Färbung der Paschenschen Körperchen durch Versilberung Zentralbl Bakt. Abt. I Orig. 100 385.

Nohrega, P., and Reis, A. S.: 1919. Preparação de vacina contra a bouba aviária com virus de pombo purificado pela penicilina e cultivado na cavidade cório-alantóide de embrião de pinto. Arch. do Instituto Biologica, São Paulo, Brazil. 19:23.

Prier, J. E.: 1951. Experimental immunization of chickens with combined whole embryo New-castle and fowl pox vaccines. Vet. Med 46:103.

Reis, J., and Nobrega, P.: 1937. Sobre um virus tripathogenico de bouba de canario. Arch. do Instituto Biologica, São Paulo, Brazil. 8:211. Cited by Brunett (1945).

Rivolta: 1869. Cited by Goodpasture (1928) and von Reischauer (1906).

Robbins, B. H.: 1944. Effect of penicillin and patulin on fowl pox. Proc. Soc. Exper. Biol. and Med. 57:215.

Sabban, M. S.: 1954. Fowlpox and the use of the whole embryo vaccine in controlling the disease in Egypt. Am. Jour. Vet. Res. 15:133.

Seegar, K. C., and Price, R. J.: 1956. Evaluation of immunity to fowl pox. I. Immunization of young chicks with pigeon- and fowl pox vaccines. Poultry Sci. 35:372.

Sevoian, M.: 1960. A quick method for the diagnosis of avian pox and infectious laryngotracheitis. Avian Dis. 4:474.

Siegel, D. V., and Leader, R. W.: 1957. Comparative histopathology of skin reactions in the chicken, turkey, and canary infected with a strain variant canary pox virus. Am. Jour. Vet. Res. 18:183.

Stafseth, H. J.: 1931. Pigeon-pox in Michigan. Jour. Am. Vet. Med. Assn. 79:822.

Stuppy, C.: 1932. Uebertragung von Geflügelpocken durch Mücken. Deutsch. tierarzt Wochenschr. 40:260. Cited from Natl. Abst. (1934)

Syverton, J. T., and Cowan, I. M.: 1944. Bird pox in the sooty grouse, Dendragapus fuliginosus fuliginosus with recovery of the virus. Am. Jour. Vet. Res. 5.215.

le Heunepe, B. J. C.: 1926. Thèse pour le Doctorat Vétérinaire. Cited by Doyle and Minett (1927).

——— 1927 Combating poultry diseases by the State Serum Institute. Data from six thousand autopsies Rep Proc Third World's Poultry Cong., p. 261.

Thorning, W. M., Graham, R., and Levine, N. D.: 1943a. Studies on certain filtrable viruses, IV. Immunogenic properties of fowl pox virus prepared from the entire embryo. Poultry Sci 22 287.

——— Graham, and Levine, N. D: 1943b. Studies on certain filtrable viruses. V. The immunogenic properties of the entire chick embryo inoculated with fowl-pox virus. Am. Jour, Vet Res 4.250

van Rooyen, C E · 1954 A revision of Holmes's classification of animal viruses, Suborder IJI (Zoophagineae) Canad Jour Microbiol. 1:227.

von Reischauer, O.: 1906 Ueber die Pocken der Vögel ihre Beziehungen zu den echten Pocken und ihren Erreger Zentralbl, Bakt. Abt. I, Orig. 40:356.

Ward, A. R., and Gallagher, B A : 1920. Diseases of Domesticated Birds. The Macmillan Co , New York. P, 96

Woernle, H : 1963. Agar-Diffusionsverfahren und Virusinfektionen des Huhnes. Proc. 17th World Vet. Cong. 2·1423.

Woodruff, A. M., and Goodpasture, E. W.: 1931. The susceptibility of the chorio-allantoic membrane of chick embryo to infection with the fowl-pox virus. Am, Jour. Path. 7.209.

Woodruff, C. E, and Goodpasture, E. W.: 1929. The infectivity of isolated inclusion bodies of fowl-pox. Am Jour. Path. 5:1.

——— and Goodpasture, E. W.: 1930. The relation of the virus of fowl-pox to the specific cellular inclusions of the disease. Am. Jour. Path. 6:713.

Zargar, S. L., and Pomeroy, B. S.: 1950. Isolation of Newcastle disease virus from commercial fowlpox and laryngotracheitis vaccines. Jour. Am. Vet. Med. Assn. 116:304.

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27

Fowl Plague

Fowl plague (fowl pest, *peste aviaire*, *Geflügelpest*) is an acute, highly infectious, generally fatal virus disease of fowls and sometimes of water birds.

History. Fowl plague was described first by Perroncito (1878) in Italy, and later studied by Rivolta and Delprato (1880), who found it to be different from fowl cholera and called it *typhus exsudativus gallinarum*. Subsequently, it was discovered in enzootic form in southern Europe and has been observed in many countries of the world. It has been widespread in Austria, has been found in Switzerland, Rumania, and Russia, and occasionally has spread to France, Holland, and Great Britain. The disease is indigenous in Egypt, has extended widely in Asia, particularly in China and Japan, and has occurred also in South America.

Fowl plague was reported first in North America in 1924–25 and again in 1929. The first severe losses appear to have occurred in the poultry market of New York City, and others later in the poultry markets of New Jersey and Philadelphia. Dr. John R. Mohler, Chief of the United States Bureau of Animal Industry, reported the existence of the disease in the United States in December, 1924. Its occurrence in New York was reported by Brunett (1925) and in Pennsylvania by Stubbs in the same year. Later in 1925, Beaudette reported the presence of fowl plague in New Jersey; Julien, in Indiana; Boughton and Tunnicliff, in Illinois; and Johnson, in Michigan. An outbreak, reported by Beaudette and associates in New Jersey in 1929, was confined to a few flocks in one locality and was eradicated promptly.

Etiology. Centanni and Savonuzzi (1900) demonstrated the cause of fowl plague to be a filterable virus, and later their results were confirmed by other workers. Weineck (1940) reports that the infectivity of tissues containing fowl plague virus is destroyed by extraction with alcohol ether,

alcohol, benzene, and chloroform but not by acetone or ether. Weineck considers this as evidence that the virulent component of the virus is lipoid in nature. The disease attacks chickens and related species, but chickens and turkeys are found affected most frequently and are considered most susceptible. With few exceptions, which seem to be immune, chickens are infected easily by subcutaneous, intramuscular, intraperitoneal, or intravenous injections of amounts even as small as one millionth of a cubic centimeter. Feeding infection also succeeds. Introduction of the virus through injuries to the skin or by instillation into the eye produces the disease. After such infection, chickens die in 36 to 72 hours and may or may not show symptoms.

Natural infection among pigeons is not found so commonly, water birds, such as ducks and geese, often remain free when chickens are attacked severely. It is a curious fact that this disease is confined frequently to a single species, and that other fowls on the same premises are not infected. Artificial infection, even by large amounts of virus from the species in which it has occurred naturally, often fails when injected into other species. Thus, on premises where there is great mortality among chickens, waterfowl usually are resistant. Injection of the virus into the central nervous system succeeds more frequently among pigeons and water birds, in which case the disease usually gives rise to nervous symptoms, such as convulsions and paralysis.

Mammals are considered immune, as artificial infection has been unsuccessful, and it is believed that this disease is not dangerous to man, as there are no recorded cases. Morcos (1946) reports the successful transfer of the virus of fowl plague from birds into mammals by using defibrinated infected fowl blood injected subdurally into white mice in which the virus became fixed after the fifth passage. The intracerebral injection was made just above and posterior to the eye, and after the fifth passage produced death in about 3 days. At the same time, the virus became attenuated in fowls with a period of incubation of 6 days instead of the previous 3 day period. The mouse brain virus treated with ether was antigenic, and Morcos believes it is promising as an immunizing agent.

Symptoms. Fowl plague appears with sudden onset. Chickens may die without showing any symptoms. Usually there is weakness and an inclination to stay on the roost or in some secluded place to avoid disturbance. Dullness and inappetence are present. Hens stop laying, the feathers are ruffled, and the birds stagger (Fig. 27.1).

Cyanosis develops, with the comb and wattles becoming dark red or blackish. The eyes are dark, the eyelids close, the conjunctiva is red and swollen. There is fever, the body temperature rising to 110°-112° F., and gradually lowering until it is subnormal (100°-103° F.).

Edema of the head, consisting of an exudation of serum into the subcutaneous tissues, marked around the eyes, ear lobes, and wattles, with a tendency to extend

FIG. 27.1 — Fowl plague. Dullness, listlessness, ruffled feathers.

downward along the throat toward the breast, frequently appears. Edema of the glottis may develop, followed by difficulty in respiration. The chickens may open their beaks for air and breathe with a rattling sound; suffocation may occur (Fig. 27.2). Mucus exudes from the nostrils, with a gray or reddish, blood-tinged exudate, which may cause the chickens to shake their heads in an effort to expel the obstructive discharge. Similar exudate may be found in the pharynx, which may contribute to the gasping and rattling sounds. The mucosa of the mouth may show small hemorrhages, and fibrinous exudate may be observed. Diarrhea also may be present, usually profuse and watery. Finally, the head cannot be raised from the ground, coma develops, the respiration becomes more labored, and death results, usually within 2 days.

Many modifications of symptoms may be noticed. Nervous disorders frequently are associated, particularly in cases that do not die early, with excitation, convulsions, rolling, or circling movements. There also may be ataxia and blindness.

In 1946, Jungherr and associates reported that in experimental work with the Dutch East Indies strain of virus, the first symptoms were usually observed within 18 to 50 hours after inoculation with 100 or more minimal lethal doses of virus. Death occurred within the next 24 hours. The first evidence of the disease was a decrease of sensitivity to sensory stimuli. There was a pronounced general malaise, congestion of comb and wattles, and closing of the eyes, with the head resting on the breast or on the floor of the cage. Loss of appetite was evident, while the desire to drink remained, so that birds frequently would fall asleep while drinking and let the water run from the mouth. Whether birds recovered or whether death occurred later, small focal or confluent areas of necrosis often were found on the comb and wattles. Inappetence, marked dehydration, loss of flesh, and in some cases severe torticollis with starvation were found. The corneas showed focal or diffuse opacities in rare cases.

Pathology. The virus of fowl plague is present throughout the body and in the blood, the nervous system, all the tissues and tissue fluids, is secreted from the glands, and is found in the nasal and oral secretions, the intestinal and urinary excretions, and in the mucous and serous exudates. The virus, or materials containing it, is highly infectious in very small amounts; and the blood is so rich in virus that 0.000,000,1 cc. may be infectious for the chicken. The virus appears to be in close contact with the blood cells, while plasma or material free of cells is less infectious. It is killed easily; exposure to direct sunlight or to a temperature of 70° C. for a few minutes renders it inactive. Moses et al. (1947) report the virus is destroyed within one hour at pH of 4.0. Its activity is destroyed quickly by the common antiseptics and disinfectants. Cold, however, promotes longevity of the virus, and it may be preserved for long periods under refrigeration. Desiccation or glycerination preserves the virus so that it retains its virulence for years. Blood from chickens suffering from the disease, preserved in sealed test tubes, remains infectious for a long time. Filtrates retain their virulence with less uniformity than unfiltered material. Purchase (1931) showed that the virus of fowl plague re-

FIG. 27.2 — Fowl plague. Chicken's head, swollen about eyes, wattles, and ear lobes.

tained its activity in flesh for 237 days, and in the bone marrow for 303 days when kept at chilling temperature. He believes the disease may be spread by feathers, since he found that the virus survived on feathers for 18 days after being plucked from a chicken dead of fowl plague. Burnet and Ferry (1934) have reported the propagation of the virus by inoculation of the chorio-allantoic membranes of developing chicken embryos. Jungherr et al. (1946) studied the inoculation of the Dutch East Indies strain into embryonating chicken eggs. Death was caused, and, regardless of age or route of inoculation, bright to dark red discoloration indicative of congestion of the embryonic tissues, especially the skin and less so of the musculature, occurred. Scattered punctiform hemorrhages were found in the skin and skeletal musculature with renal congestion in 12-day-old or older embryos. Minimum doses of virus in some eggs caused slower death of embryos, which showed tumefaction and sometimes pinhead-sized, gray focal areas in the spleen and rarely in the liver. Variant viruses were obtained from such spleens which, in further egg passages, produced congestive and hemorrhagic lesions similar to the parent strain.

Histopathological studies were made on embryonating chicken eggs. Inoculation into the allantoic sac usually failed to show any specific lesions. Inoculation of the virus onto the chorio-allantoic membrane was followed in about one-fourth of the cases by shallow hemorrhagic ulcers in the ectoderm, filled with disintegrated red cells and heterophils, and delimited by an intensely congested and fibrotic zone of the mesoderm. The lesions in the embryo were multiple capillary hemorrhages, particularly in the skeletal muscles and in the spinal cord and brain as well as the myocardium and the gizzard wall.

Hotz and Schafer (1955), using thin histologic sections of allantoic membranes from infected chicken embryos in the electron microscope, demonstrated deviations from the normal picture beginning 4–5 hours after infection. At the sixth hour,

particles resembling elementary bodies were observed at the cell surface, but not within the cell.

Shinnin et al. (1957) report serial propagation and cytopathogenic effect of fowl plague virus is the same in cells of pig kidney as in cells of chicken embryos.

Waterson (1958), reports fowl plague virus in agar suspensions of chicken embryo cells form plaques in numbers directly proportional to the quantity of the inoculum. The plaque count is not affected by allowing time for adsorption of virus before mixing with the agar.

Franklin (1958), using fluorescent antibody technique, studied the growth of fowl plague virus in cultures of macrophages derived from chicken leukocytes and found a soluble antigen multiplying in the nucleus. These studies indicated that soluble antigen does not multiply in cells undergoing mitosis. Hemagglutinating antigens were found only in the cytoplasm and were detected later than the soluble antigen.

The changes found at postmortem examination are those of septicemia. The lesions cannot be depended upon entirely for diagnosis but are somewhat characteristic and usually quite uniform. Rigor mortis sets in early and is complete. There is cyanosis of the head; the face, comb and wattles are dark; and the conjunctiva is swollen, often petechiated. The nostrils and beak show accumulation of mucus, frequently stained or streaked with blood. There may be edematous swellings of the head; and the fluid, clear and straw-colored, may be most marked in the face, about the eyes, in the ear lobes, the wattles, or in the subcutaneous tissue of the neck and breast (Fig. 27.3).

Removal of the skin shows engorgement of all blood vessels. The flesh is red. Hemorrhages are widespread and vary from the smallest possible petechiae to ecchymoses. Those that are small and widely scattered may be overlooked easily but are conspicuous when grouped, as in the proventriculus or over the abdominal fat. They may be found in any tissue, but are

FIG. 27.3 — Fowl plague. Chicken's head showing swollen wattles.

frequently in the muscles of the breast. Very distinct petechial hemorrhages, quite characteristic and appearing to have been sprayed on with an atomizer, are found on the inner surface of the sternum when the breast is removed. Hemorrhages usually are found in the fat about the abdominal cavity, and petechiae frequently are sprinkled over the fat tissue forming the bottom of this cavity. Petechial hemorrhages also are particularly noticeable in the abdominal fat over the proventriculus, gizzard, and mesentery, and in the thoracic fat over the heart.

The most characteristic lesion is the hemorrhagic alteration in the proventricu-

lus, which can be seen after the whitish mucus is washed off the mucous membrane. Such hemorrhages, usually ecchymoses, may be observed between the conical elevations or secreting glands of this portion of the stomach and are more noticeable when they occur as bright red blotches on the mucous membrane where it becomes smooth on entering the gizzard (Fig. 27.4).

Petechiae or ecchymoses also are seen in the gizzard after the rough membrane or cuticle has been removed. The intestine frequently shows hemorrhagic changes, especially in the duodenum. These hemorrhages, petechiae or ecchymoses on the serous or mucous coat, are accompanied by catarrhal enteritis. The hemorrhagic enteritis usually present in fowl cholera is not found in fowl plague and may be helpful in differentiating these diseases.

The liver, spleen, and lungs do not show much change. Congestion may be found, and also hemorrhagic fluid in the peritoneum and pericardium. Exposure to the air results in clotting, and in some cases fibrinous exudate is already present. This is spoken of sometimes as the exudative form. The ovary, when functioning, shows highly engorged blood vessels, especially in the large follicles. The oviduct often shows gray exudation, and the wall is swollen.

Microscopically, the chief changes are congestion and hemorrhages. Perivascular round-cell infiltrations have been noted. Some have described necrosis in brain tissue and the presence of bodies which

FIG. 27.4 — Fowl plague. Hemorrhages of proventriculus (chicken).

strongly resemble intracellular inclusions.

Beaudette and associates, reporting the outbreak of fowl plague in New Jersey in 1929, recorded the occurrence of vesicles on the comb and wattles of chickens artificially infected. These vesicles were observed in cases that ran longer than the usual course and varied in size from a pinhead to 4–5 mm. in diameter. The same investigators also reported the occurrence of edema of the feet and tibiometatarsal joint, and spots of violet color on the scales of the shanks and feet, some as mere spots and others as blotches 4–5 cm. in length.

Jungherr et al (1946) in experimental work with the Dutch East Indies strain reported a variety of postmortem changes. The acute cases showed congestive, hemorrhagic, and transudative changes. Congestion was evident in the skin, the comb and wattles, musculature, oropharynx, larynx, trachea, and the abdominal viscera. Punctiform hemorrhages were most often found in the coronary fat beneath the epicardium, especially the left auricle and around the roots of the large vessels. Intestinal hemorrhages were discrete, scattered along the serosa in the walls of the intestine and in the mucosa of the proventriculus, and in the region of the Peyer's patches and the cecal tonsils. Transudative changes were less frequent and showed moderate to severe edema of the lungs with congestion or hemorrhage and consolidation. Pericardial fluid that jellied on exposure to the air was sometimes found, and in a few cases subcutaneous edema of the hocks, feet, breast, neck, and head was present. Usually the spleen appeared small and anemic, often almost white except for stellate areas of capillary injection near the mesenteric attachment.

Jungherr et al. also studied the histopathological changes in over 100 experimental cases of different ages while experimenting with the Dutch East Indies strain. They considered the basic lesion roundish but not sharply delimited, foci of necrosis of various organs. The young

foci were recognized by acidophilic staining. The cellular architecture was at first undisturbed, but in well-developed foci the tissue cells were swollen and vesicle-like, with the cell membrane prominent and the nucleus small and marginated, leaving a large cytoplasmic space containing eosinophilic granules or globules. The necrotic foci did not show pyknosis or karyorrhexis, did not become confluent, but remained scattered. The foci were rarely numerous but were found in a variety of the organs such as the spleen, lung, thymus, liver, gallbladder, kidney, heart, pancreas, proventriculus, intestine, comb and wattles, iris, and occasionally in the gonads. The spleen showed the highest incidence of necrotic foci, and it was estimated that more than three-fourths of the cases showed spleen involvement, with the other organs in a falling order of frequency.

The necrobiotic foci in various organs were often accompanied by hemorrhages, congestion, and edema. Hemorrhages were found in the submucosa of the secondary pulmonary bronchi, in the alveolar tissue, in the proventricular mucosa, in the thyroid, subepicardium, myocardium, and elsewhere. The lungs often showed edema and variable degrees of congestion with some proliferation, capillary congestion, and edema, and occasionally fibrinous thrombi were found in the region of necrotic foci.

Diagnosis of fowl plague may be returned when an acute, plaguelike infectious disease resembling fowl cholera is encountered, accompanied by cyanosis and edema of the head and hemorrhages in the proventriculus, gizzard, and abdominal fat. In questionable cases, doubt is removed by negative bacteriological examinations, negative results from inoculations into mammals, and filtrates producing typical symptoms with characteristic lesions in chickens. Differentiation from other acute infectious diseases, particularly fowl cholera, is difficult. Both diseases present similar symptoms and lesions: rapid onset, cyanosis, prostration,

diarrhea, high mortality, and especially hemorrhagic alterations. The losses from fowl plague are regular and extensive, while from fowl cholera the mortality is likely to be irregular. In the recorded natural outbreaks of fowl plague in this country, where chickens are associated with waterfowl, such as ducks and geese, the latter are not affected; while in fowl cholera, ducks and geese are highly susceptible and show the disease in a marked and highly fatal form. European literature indicates that young geese sometimes are affected. In numerous necropsies of fowl cholera cases, areas of focal necrosis appearing as whitish-yellow points are observed scattered over the liver. Such changes have not been found in fowl plague. The hemorrhages of fowl cholera more often are confined to the heart and intestine, while in fowl plague the hemorrhagic lesions are more likely to be scattered throughout the body.

Placidi and Santucci (1956) report fowl plague virus agglutinating the erythrocytes of the chicken, camel, horse, donkey, and mule.

Grausgruber (1958) reviewing the various serologic methods of diagnosis, advocates the agglutination test, using fowl erythrocytes sensitized by suspensions of infected organ material. He states this gives the most reliable results in dead birds.

In cholera, culture examination is positive; inoculation into rabbits, mice, and pigeons produces death, and filtrates do not cause the disease. Where there is recourse to laboratory procedure in fowl plague, culture examination and inoculations into rabbits, mice, and pigeons are negative, while filtrates will produce the disease in chickens.

Newcastle disease may be found in chicks, in growing chickens, and in mature fowl. The disease in chicks frequently begins with gasping, wheezing, or coughing, and the same changes are also found in older birds. It spreads very rapidly and may go through an entire group in 1 or 2 weeks. Later many different kinds of nervous symptoms and paralysis may

be found. Shivering, incoordination, convulsions, and chronic spasms of the head or neck and body have been noted. Twitching of the head or tail may be seen, with birds walking in circles, forward or backward. Alternating periods of excitation and depression may be found. Birds may stand motionless with the head drawn back and eyes fixed. The head may be drawn toward the ground. The presence of respiratory difficulty followed by nervous symptoms points to Newcastle disease. Mortality may be high in young birds and slight or none in mature birds. Layers show an abrupt drop in egg production followed by irregular shells, discolored shells, and soft shells. Varying lengths of time are required for resumption of production.

Differential diagnosis. Doyle in England has described a disease similar to fowl plague from which it is difficult to differentiate. He discovered the malady near Newcastle and named it "Newcastle disease." It also has been described in other places as "pseudo-fowl plague." The symptoms are quite similar, and there is high mortality, but the pathologic changes are not nearly so marked. It can be transferred readily by blood, brain, organ emulsion, oral discharges, and feces, as well as by filtrates of these materials. The hemorrhages present in this disease are not nearly so marked as in fowl plague, and the period of incubation is longer (about 1 week or more). Experimental infection by contact often does not succeed in fowl plague, whereas in Newcastle disease contact infection appears easier.

Apoplectiform septicemia and sleeping sickness cause symptoms and lesions similar to fowl plague but can be differentiated by the demonstration of a streptococcus in the blood stream. These diseases produce depression, staggering, prostration, coma, and death. Postmortem examinations disclose hemorrhages and hemorrhagic discolorations which are rather widespread. There may be lung congestion and hemorrhage, and usually a hemorrhagic pericarditis. Peritonitis is

frequent and also catarrhal or hemorrhagic enteritis.

Phosphorus poisoning produces hemorrhagic lesions in the proventriculus and may be confused with fowl plague. Phosphorus, highly poisonous to chickens, causes depression, weakness, trembling, thirst, and sometimes diarrhea, and may result in sudden death. Postmortem examinations may show hemorrhages in the proventriculus, usually with erosions, and extending more deeply into the tissue than in fowl plague. In phosphorus poisoning there is usually severe enteritis, particularly in the upper portion of the intestine. If such poisoning is suspected, attention should be directed to the detection of the phosphorus vapor that may be noticed as a transient cloud when the crop, proventriculus, and gizzard first are opened. The contents also have the distinctive odor of phosphorus, and if such material is taken into a dark room or mixed with dilute acid, the characteristic phosphorus luminosity is seen.

Botulism may be attended by sudden onset and cause high mortality. It may attack a flock with overnight suddenness, but the clinical picture is so striking that it should not be confused with any other disease. Usually no lesions are found in botulism.

Edema of the wattles, usually an infection of one or both wattles, with listlessness, inappetence, and marked depression, may be confused with fowl plague. Some cases show a slight swelling, while in others the wattles become enormous and occasionally rupture. The swelling first contains an edematous fluid which gradually thickens and becomes caseated. The mortality is not high unless the disease reaches the sinuses or spreads systemically. It is frequently due to the organism of fowl cholera.

Prognosis. The course of fowl plague is quite rapid in chickens, which often live only a few hours. After artificial injection the fowls usually die in 36 to 72 hours. Death frequently takes place after a short struggle, and the victim often is found dead on its back. Occasionally recovery occurs, and such survivors are solidly immune.

Epizootiology. The virus is found in many European countries, and the disease occurs where conditions are favorable for it. Fowl plague may be spread rapidly under certain conditions and usually disappears after a time, due in part to its high mortality; thus the disease is somewhat self-limiting.

The diseased bird is the most dangerous factor in the spread of the disease, so the introduction of one or more infected chickens may cause an outbreak. Usually the newly purchased birds die first, although this is not always the case, since carriers are known to exist. Losses begin within 1 or 2 days after the disease has been introduced.

The virus is present in the eye, nasal secretions, in the mouth, feces, and urine. The feed, drinking water, and soil become contaminated, and the virus may be spread on the shoes of attendants. Livestock dealers provide excellent opportunity for the spread of the disease as they travel from place to place. Chickens may ingest the virus with contaminated feed or water and with substances picked up from the soil, while the virus also may enter through the respiratory tract. The virus is present in the blood, so many believe vectors play a part in the natural spread of the disease. It is a commonly held opinion that susceptible chickens in close association with infected chickens often do not contract the disease, but when injected frequently succumb. This lends support to the belief that vectors are instrumental in transmitting fowl plague. It is also the common experience that healthy chickens placed in uncleaned, undisinfected cages in which others have died of fowl plague frequently do not contract the disease. This is attributed to rapid destruction of the virus except under conditions favorable to its existence.

Wild birds and semiwild birds that commonly associate with farmyard fowls also may spread the disease. Thus, pigeons, sparrows, and similar birds under some circumstances disseminate the di-

ease. Fowl plague may also be spread by streams. In certain instances it has been noted that chickens on farms downstream from infected areas have contracted the disease, presumably from the water.

Immunization. Fowl plague is highly fatal. The few birds that recover appear to be solidly immune. The serum from recovered chickens shows serum neutralizing and hemagglutinating inhibiting antibodies and will protect susceptible birds, but only for a short time, and is impractical. Generoso and San Agustin (1947) report a vaccine of fairly good protective power against Philippine avian plague using oils and saponin solution as vehicles. Daubney et al. (1949) state "no very great success has hitherto attended the efforts of workers to devise a satisfactory technique for artificial immunization." Their experiments indicated that adjuvants and the use of killed acid-fast bacilli, together with formalized vaccines, seemed to enhance immunization. These workers stated that a strain was found that when passed through pigeon embryos was apathogenic for chickens, but gave solid immunity against virulent fowl plague virus.

Hallauer and Kronauer (1960) report variants of fowl plague virus isolated from human explants (amnion, HeLa, KB cell strains) showed a high degree of attenuation, allowing inoculation by various routes of maximum doses without danger to the bird, and excellent immunizing activity.

Control and eradication. Outbreaks should be reported immediately to livestock sanitary authorities. Poultrymen should be warned against the addition of fowls to flocks. If additions are necessary, the added fowls, regardless of source, should be isolated until proved healthy. Sick fowls should be destroyed, carefully examined, and carcasses burned or properly buried. Frequent, diligent cleaning of premises, coops, crates, and carriers, followed by thorough disinfection, is essential. Flückiger (1950), reporting for the International Commission for the Control of Fowl Plague, recommends that fowl plague be a reportable disease in all countries and that all birds on infected farms be destroyed.

Outbreaks of fowl plague in the United States have always been eradicated with methods designed to limit and destroy the disease. In many instances the disease was self-limiting, inasmuch as entire flocks succumbed, leaving no survivors. Since the disease spreads most easily and rapidly through the intermingling of fowl, exposure to infected premises, coops, crates, and other containers and carriers, the best procedure is the destruction of all birds in the infected flock and the disinfection of houses and equipment.

It is fortunate that the outbreaks of fowl plague in the United States have been recognized and measures for control instituted promptly. It is perhaps the most fatal of fowl diseases, capable of causing such destruction to the poultry population as to be of economic importance in diminishing the food supply. The dangerous character of the disease has warranted the radical methods employed in each outbreak so that complete eradication was effected within a few months. Quarantines were imposed, embargoes placed, and poultry shipping restricted. Slaughter, sanitation, and disinfection aided in the control program.

Federal disease restrictions were applied to the poultry industry for the first time in the United States during the 1924–25 outbreak. Federal and state employees supervised the cleaning and disinfection of 2,718 plants, 8,140 cars, 352,525 coops, and 124,997 pieces of miscellaneous equipment.

REFERENCES

Beaudette, F. R.: 1925. Observations upon fowl plague in New Jersey. Jour. Am. Vet. Med. Assn. 67:186.
——— Hudson, C. B., and Saxe, A. H.: 1934. An outbreak of fowl plague in New Jersey in 1929. Jour. Agr. Res. 49:83.

Boughton, I. B., and Tunnicliff, E. A.: 1925. European fowl pest in Illinois. Jour. Am. Vet. Med. Assn. 67:183.

Brunett, E. I.: 1925. The occurrence of a disease of chickens in New York State caused by a filtrable virus. Jour. Am. Vet. Med. Assn. 66:497.

Burnet, F. M., and Ferry, J. D.: 1934. The differentiation of the viruses of fowl plague and Newcastle disease. Experiments using the technic of chorio-allantoic membrane inoculation of the developing egg. Brit. Jour. Exper. Path. 15:56.

Centanni and Savonuzzi: 1900. Cited by Gerlach, 1929, Kolle and Wass. Path. Mikr. 9:165.

Daubney, R., Manil, V., and Zahran, G.: 1949. Vaccination against fowl plague. Jour. Comp. Comp. Path. and Therap. 59:1.

Doyle, T. M.: 1927. A hitherto unrecorded disease of fowls due to a filter-passing virus. Jour. Comp. Path. and Therap. 40:144

Flückiger, G.: 1950. Internationale Bekämpfung der Geflügelpest. Schweizer Arch. für Tierheilk. 92 657.

Franklin, R. M.: 1958. The growth of fowl plague virus in tissue cultures of chicken macrophages and giant cells Virology 6.81.

Freese, Dr.: 1908. Fowl plague, with special reference to its pathological anatomy. Trans. from the Deutsch. tierärztl. Wochenschr., 1908, 175. Jour. Comp. Path. and Therap. 21:212.

————: 1925. Fowl pest with special consideration of the pathology of the disease. Trans. by L. P. Doyle. Jour. Am. Vet. Med. Assn. 67:203.

Generoso, J. D., and San Agustin, F.: 1947. Some studies on avian pest immunization. Philippine Jour. Anim. Ind. 9:75.

Grausgruber, W.: 1958. Biological and serological diagnosis of Newcastle disease and fowl plague. Wien. tierärztl. Monatschr. 45:76.

Halliever, C., and Kronauer, G.: 1960 Immunization experiments with experimentally-induced variants of classical and atypical fowl plague viruses Arch. ges. Virusforsch. 10:46.

Hotz, G., and Schafer, W.: 1955. Ultrahistologic study of the multiplication of fowl plague virus. Zeitschr. Naturforsch. 10b:1.

Hutyra, F., Marek, J., and Manninger, R.: 1938. Special Pathology and Therapeutics of the Diseases of Domestic Animals. Alexander Eger. Chicago, Vol. 1.

Johnson, S. R.: 1925. European fowl pest in Michigan. Jour. Am. Vet. Med. Assn. 67:195.

Julien, R. C.: 1925. Fowl pest in Indiana. Jour. Am. Vet. Med. Assn. 67:178.

Junghern, E. L., Tyzzer, E. E., Brandly, C. A., and Moses, H. E.: 1946. The comparative pathology of fowl plague and Newcastle disease. Am. Jour. Vet. Res. 7:250.

Komarov, A.: 1934. A study on "cell-inclusion" disease in fowls. I. On the identity of acute "cell-inclusion" disease and fowl plague. II. On the diagnostic value of the "chromatic inclusions" in the leukocytes. Jour. Comp. Path. and Therap. 47:282, 296.

Kunst, H.: 1949. The differences between Newcastle disease and fowl plague. Tijdschr. v. Diergeneesk 74.403.

Lerner and Wojtek. 1942. Huhnerpirochätose. Deutsch. tierärztl. Wochenschr. 50:364. (Abst. Vet. Bull. 13:51)

Matzke, M.: 1942. Die Diagnose der Hühnerpest. Zeitschr. Infekt-Krankh. parasitäre Krankh. u. Hyg der Haustiere 59:42 (Abst. Biol. Abst. (1943) 17, No. 20580, p. 1948)

Mohler, J R 1924. Statement from Bureau of Animal Industry.

————: 1926 Fowl pest in the United States. Jour. Am. Vet. Med. Assn. 68:549.

Moreos, Z.: 1946 Fowl plague in Egypt. Immunization mouse neurotropic fixed virus. Vet. Jour. 102:3.

Moses, H. E., Brandly, C. A., and Jones, E. E.: 1947. The pH stability of viruses of Newcastle disease and fowl plague. Science 105:477.

Perroncito: 1878 Cited by Gerlach, 1929, Kolle and Wass. Path. Mikr., p. 165.

Placidi, L., and Santucci, J.: 1956. Agglutination of erythrocytes of the hen, camel, horse, donkey and mule, by Newcastle disease virus, and by fowl plague virus (transl. title). Ann. Inst. Pasteur 90.528.

Purchase, H. S.: 1931. Experiments on the viability of the virus of fowl-plague under trade conditions. Vet. Record 11 644.

Reis, J., Nobrega, P., and Reis, A. S.: 1956. Tratado de Doencas das Aves. Instituto Biologica, São Paulo, Brazil. P. 468.

Rivolta and Delprato: 1880. Cited by Gerlach, 1929, Kolle and Wassermann. Path. Mikr. Vol. 9, p. 165.

Shimizu, T., Ishizaki, R., Kono, Y., Ishii, S., and Matumoto, M.: 1957. Multiplication of Newcastle disease and fowl plague viruses in swine kidney tissue culture. Jap. Jour. Exper. Med. 27:181.

Stubbs, E. L.: 1925. Fowl plague. Univ. of Pa. Quart. 29.

————: 1925. Fowl plague in Pennsylvania. Jour. Am. Vet. Med. Assn. 67:180.

————: 1926. Fowl pest. Jour. Am. Vet. Med. Assn. 68:560.

————: 1946. Newcastle disease in Pennsylvania. Univ. of Pa., Bul. 46:3.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart.

Waterson, A. P.: 1958. Some factors affecting the formation of plaques by fowl plague virus in chicken embryo cells. Arch. ges. Virusforsch. 8.115.

Weineck, E.: 1940. Ueber die Protein-Lipoid-Simplexnatur des Huhnerpestvirus. Zeitschr. f. Immunitätsforsch. 98:463. (Abst. Vet. Bull. 12:278.)

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28

Infectious Synovitis

Infectious synovitis is recognized as a separate disease entity of chickens (Olson *et al.*, 1954; Wills, 1954a) and of turkeys (Snoeyenbos and Olesiuk, 1955). The disease is observed primarily in growing birds 4 to 12 weeks of age in the broiler-growing areas of the United States. It has been reported from England (Carnaghan, 1959), Canada (Bigland and Brown, 1955), Norway (Badstue, 1961), Germany (Burtscher, 1961), France (Guillon *et al.*, 1963), South Africa (Cole, 1964) and is probably worldwide. The morbidity is variable with reports of 2 to 75 per cent. Mortality usually is low and ranges from less than 1 to 10 per cent. Successive flocks on the same farm, following an outbreak, generally do not show the infection. The infection spread slowly. Kerr *et al.* (1963) found birds from 2 to 20 weeks old were equally susceptible. The disease has been seen in egg birds with increased frequency. On one occasion signs were not noted until the birds were in production.

Symptoms. The first observable signs in an affected flock are pale comb, lameness, and retarded growth. As the disease progresses, the feathers become ruffled and the comb shrinks. In some cases the comb is bluish-red. Swellings usually occur around the joints, and breast blisters are common. The hock joints and foot pads are principally involved, but in some birds all joints become affected. However, birds are occasionally found with a generalized infection but not having apparent swelling of the joints. The birds become listless, dehydrated, and emaciated (Fig. 28.1). Although birds are severely affected, many of them continue to eat and drink if placed near feed and water. A greenish discoloration of the droppings, which contain large amounts of uric acid or urates, is frequently seen.

Gross lesions. In the early stages of the disease necropsy reveals a viscous, creamy to gray exudate involving the synovial membranes of the joints, keel bursae, and

FIG. 28.1 — Experimental birds with infectious synovitis. Contact control bird (standing) and three typically infected birds in the advanced stage of the disease

tendovaginal sheaths (Fig. 28.2). As the disease progresses, this exudate becomes caseous. Caseous exudate is occasionally found over the skull, along the neck, and rarely extends into the muscles and air sacs. When birds become severely emaciated and dehydrated before caseous exudate develops, there is occasionally no fluid about the joints. In chronic cases the surfaces of the affected joints are frequently yellow to orange.

In the early stages of the disease, splenomegaly generally occurs. The liver is frequently enlarged, occasionally mottled, greenish or dark red. The kidneys are usually swollen, mottled and/or pale.

FIG. 28.2 — The foot of a 7-week-old turkey showing the purulent exudate in the foot pad 22 days after experimental inoculation. Similar exudates are seen in chickens.

These changes occur in approximately 50 per cent of the birds and become more pronounced and frequent as the severity of the disease increases. Even though some birds are severely affected, their internal organs appear normal. In experimental foot-pad-inoculated birds the infection frequently localizes in the inoculated foot, and no gross internal lesions are noted.

The microscopic lesions (Sevoian et al., 1958) of the brain consist of vascular endothelial thickening and adventitial proliferation in the cerebrum, cerebellum, optic lobe, degeneration of some of the Purkinje cells, and occasionally cerebellar lesions similar to those of encephalomalacia. In the liver, perivascular, periportal, and interparenchymal cellular hyperplasia of the reticular cells of the reticulo-endothelial system occur. The sinusoids are dilated and the parenchymal cells are atrophied. There is proliferation of the bile duct epithelium. The connective tissue framework of the heart, gizzard, and interlobular septa of the lungs reveals a similar reticular cell hyperplasia. Occasionally focal mononuclear infiltration and necrosis of the myocardium and a fibrinous inflammation of the pericardium are seen. A reticular cell or lymphocytic hyperplasia, or both, decrease the sinusoidal areas of the spleen. A granulocytic hyperplasia of the bone marrow occurs, and atrophy of the thymus and bursa of

Fabricius results from lymphoid degeneration in the medulla and cortex.

The embryo response (Casorso and Jungherr, 1959) to infectious synovitis is similar to that described in birds.

Giemsa stains of smears made from creamy synovial fluid reveal many large macrophage cells, lymphocytes, plasma cells, and heterophils. In many cases the heterophils predominate. No bacteria are found.

The changes in the blood components have been studied (Olson *et al.*, 1956, 1957c; Shelton *et al.*, 1957; Sevoian *et al.*, 1957). Average determinations for 31 experimentally inoculated birds were as follows: erythrocytes, 1,680,000 per mm.^{3}; leucocytes, 80,810 per mm.^{3}; hemoglobin, 6.5 gm. per 100 ml. of blood. Differential count gave the following percentages: lymphocytes, 41.9; heterophils, 31.9; monocytes, 19.3; eosinophils, 0.23; basophils, 1.1; immature leucocytes, 6.1. The thrombocyte count increased and hematocrit decreased. The gamma globulin is increased (Shelton *et al.*, 1957). The blood abnormalities increased as the severity of infectious synovitis increased, generally reaching a maximum shortly before death. The changes were most severe between the 6th and 26th day following foot-pad inoculation. In severely affected birds the erythrocytes showed anisocytosis, poikilocytosis, polychromatophilia, and achromia. Immature erythrocytes of varying degrees were present. When the birds showed signs of recovery, the blood changes showed evidence of returning to normal. Similar blood changes have been noted in experimentally infected turkeys.

Host specificity. The disease has been reported from turkeys and chickens. Pheasants and geese (Sevoian *et al.*, 1958) were experimentally infected by the intravenous route. Rabbits, rats, guinea pigs, mice, pigs, and lambs are not susceptible to experimental inoculation.

Etiology. Infectious synovitis was thought to be caused by a large particle virus or rickettsia (Wills, 1954b; Lecce *et al.*, 1955; Cover *et al.*, 1956; Olson *et al.*, 1956). Electron micrographs (Lecce *et al.*,

1955) revealed coccobacillary elements, 0.2 to 0.5μ in size, containing what appeared to be a limiting membrane. Pleuropneumonialike organisms (PPLO) (*Lecce*, 1960) were noticed growing as satellite colonies to micrococcus colonies on PPLO agar. This was confirmed by Chalquest and Fabricant (1960) who subsequently grew the organism on PPLO broth (Difco) that contained 0.1 per cent beta diphosphopyridine nucleotide (DPN), 0.1 per cent cysteine, 10 per cent heat inactivated swine serum, 0.05 per cent thallium acetate, and 1,000 units of penicillin per ml. A PPLO agar was used in a similar medium but without the cysteine, and incubation in a candle jar was required. Chalquest (1962) reported that the infectious synovitis-derived (ISD) PPLO grew better in a medium containing 0.01 per cent DPN, 0.01 per cent cysteine HCl, 0.5 per cent soluble starch, and 0.05 per cent trypticase. The ISD-PPLO fermented dextrose and maltose, but not lactose, sucrose, or mannite. Six isolates were not inhibited by hyperimmune rabbit serum prepared against *Mycoplasma gallisepticum* using *Edward's technique* (1954) as revised by Fabricant (1960). The morphology of the ISD-PPLO in Giemsa-stained smears and the colonial characteristics were similar to M. gallisepticum.

Seven broth passages and one agar passage were made before inoculation of ISD-PPLO into poults, chickens, and embryos. A typical synovitis was produced in these birds and ISD-PPLO were reisolated from swollen joints (Chalquest and Fabricant, 1960). There is little doubt that the infectious synovitis described in this report is caused by a PPLO which is serologically distinct from M. gallisepticum, the cause of CRD. This was confirmed by Olson *et al.* (1961b) and the name *Mycoplasma synoviae* proposed for the etiological agent of infectious synovitis. A large number of bacteria have been isolated from arthritic conditions in chickens and turkeys. Olson (1956) found that inoculation of the fowl pox virus into the foot pad of chickens caused the growth of the virus in the synovial

membranes with enlargement, but Newcastle disease, infectious bronchitis, or laryngotracheitis did not. Also, a synovitis has been produced experimentally by PPLO (Wasserman *et al.*, 1953), and *M. gallisepticum* have been isolated from some field outbreaks (Olson *et al.*, 1956), and from breast blisters (Domermuth, 1962).

Passage of the agent in embryonating chicken eggs, using 0.25 ml. of a 1:10 dilution of inoculum, results in mean day of death of the embryos as follows: yolk 6.7 days, amniotic 8.6 days, chorio-allantoic membrane 12 days, and allantoic 12.9 days (Lecce *et al.*, 1955). The yolk sac route of inoculation is preferable for cultivation of the agent in embryonating eggs. Those embryos which die 4 to 10 days post infection are edematous and hemorrhagic (Fig. 28.3). The hemorrhages of the skin are not obvious in those that die later. The liver, spleen, and kidneys are enlarged, and the liver is frequently mottled or contains necrotic foci. Petechiae on the chorio-allantoic membrane

(CAM) frequently appear. Plaques were produced on the CAM by two of nine isolates and by the isolate described by Thayer *et al.* (1958). Recent studies (Olson *et al.*, 1964) have established the presence of three separate entities in synovitislike conditions: (1) infectious synovitis caused by *Mycoplasma synoviae*, (2) *Mycoplasma gallisepticum*, and (3) an unidentified arthritis-producing agent.

Transmission. Low morbidity in many flocks suggests an agent that spreads slowly. This was confirmed in laboratory trials where the incubation period in contact controls was 24 to 80 days. Spread occurred more frequently to uninoculated birds when the principles are inoculated intrasinusly (Skamser and Seeger, 1960). Birds are susceptible to the respiratory route of inoculation and following such inoculation birds develop agglutinins without showing signs of infectious synovitis. This respiratory infection was not exalted to systemic disease by the exposure to infectious bronchitis (Olson *et al.*, 1964a). In
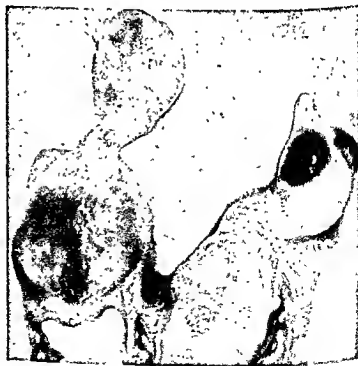
FIG. 28.3 — Two 15-day-old embryos. The infectious synovitis-inoculated embryo on the left is edematous and has numerous hemorrhages in the skin. The embryo on right is normal.

birds experimentally infected by inoculation, at 3 to 6 weeks of age, with joint exudate from infected birds or yolk from infected embryos, the order of susceptibility and incubation period is as follows: foot pad, 2 to 10 days; intravenously, 7 to 10; intracranially, 7 to 10; intraperitoneally, 7 to 14; intrasinusly, 14 to 20; conjunctival instillation, 20. Birds are also susceptible to intramuscular and intratracheal inoculation. The incubation period varies with the amount and pathogenicity of the inoculum. Sevoian *et al.* (1958) reported an incubation period as short as three days in intravenously inoculated birds. The agent has been found in nearly all body tissues but not in bile or intestinal contents (Cover and Benton, 1957). In intravenously inoculated birds the blood was infective by the eighth hour but not by the fourth. In intramuscular inoculated birds the blood was infective by the forty-eighth hour but not by the thirty-second hour. The agent was present in the blood until the fifteenth day. The duration of the viremia is not known but it was not present at 52 days after inoculation (Benton and Cover, 1959). Passage in eggs prolongs the incubation period and reduces the pathogenicity of the agent. Direct contact is necessary for infection. When a wire partition separates pens of chicks, spread does not occur. Vertical transmission is suspected. Chicks from hens that survived a natural outbreak and chicks from hens that were experimentally inoculated were brooded until 5 to 10 weeks of age. One of the 543 chicks developed synovitis at 3 weeks of age (Wills and Delaplane, 1955). The disease has been seen in chicks 6 days old (Thayer *et al.*, 1958), further indicating fertile egg transmission. Isolation of the agent was reported from 2 dead embryos one of which was laid as early as 48 hours and the other 11 to 29 days after inoculation of the dams (Snoeyenbos and Basch, 1958). Carnaghan (1961) found infection in 6 per cent of the embryos and in one chick hatched from eggs produced by clinically normal survivors of the disease. He also experimentally produced the disease in adult birds. The agent was isolated from embryos dying during incubation and from chicks hatched from eggs produced by survivors of the experimental disease. In view of these observations and the PPLO etiology of infectious synovitis there is little doubt that egg transmission is the most likely means of transmission. *M. synoviae* occurs in the respiratory tract of birds without producing clinical signs or gross lesions (Olson *et al.*, 1964a).

Diagnosis. The presence of pale comb, droopiness, emaciation, leg weakness, along with breast blister and enlarged foot pads, or hock joints which contain a viscous, creamy or caseous exudate, with splenomegaly and enlarged liver or kidneys, is sufficient to make a presumptive diagnosis. A positive diagnosis may be made by isolation and identification of the mycoplasma involved. The serum plate agglutination test using *M. synoviae* antigen and the procedure as for CRD may be used to make a positive diagnosis. However, it takes approximately two weeks for agglutinins to develop in chickens (Olson *et al.*, 1965). Cross reactions will occur between *M. synoviae* and *M. gallisepticum*; therefore, it is usually necessary to find the end point of reactivity of the serum (Olson *et al.*, 1965). Bacteria as a cause of synovitis or arthritis must be eliminated by bacteriological procedures. Embryonating chicken eggs or chickens or both should be inoculated as a further check since primary isolation of mycoplasma is frequently difficult.

Immunity. Cassidy and Grumbles (1959) were not able to demonstrate immunity to IS, however, Wichmann *et al.* (1960) found that after 9 passages in tissue culture the pathogenicity was reduced but not its antigenicity. On the other hand, hyperimmune rabbit serum prepared against ISD-PPLO inhibits their growth (Chalquest and Hallfhill, 1962). The immunity to IS needs further clarification. Birds exposed intranasally were resistant to subsequent footpad challenge. (Olson *et al.*, 1964a).

FIG. 28.4 — An isolated control turkey (right) and two experimental turkeys with infectious synovitis (left).

Shelton (1958b) found 200 grams per ton of feed of CTC effective in preventing signs and recurrence of the disease if given at time of inoculation of day-old chicks. If the disease was allowed to progress for 8 days and CTC fed for 3 weeks, 200 grams per ton of feed prevented signs of IS as long as it was given but even 1000 grams per ton did not prevent signs from developing after withdrawal of medication.

An agent recovered from the joints of chickens was not susceptible to antibiotics in ova (Cover et al., 1956) and in vivo (Olson et al., 1957b). Also Snoeyenbos et al. (1958) reported strain differences in their susceptibility to antibiotics.

The reported efficacy of NF-180 (Cosgrove, 1957) has not been confirmed by other published work.

Dihydrostreptomycin injections (25 mg. per lb. body weight) satisfactorily controlled infectious synovitis if given at the time of experimental inoculation (Munro et al., 1956). If given 4 or more days after inoculation, only slight benefit was noted. Increasing the dose to 200 mg. per lb. of body weight increased its effectiveness slightly (Shelton and Olson, 1957–58; Snoeyenbos et al., 1958).

Infectious synovitis in turkeys. The disease in turkeys has not been studied to the same extent as it has in chickens. A staphylococcus arthritis is more frequently encountered in turkeys than is infectious synovitis. However Chalquest and Fabricant (1960) isolated PPLO and a staphylococcus from the same turkey.

Infectious synovitis generally causes the same signs and lesions in turkeys as in chickens (Snoeyenbos and Olesiuk, 1955). However, in turkeys the disease appears to be less acute, and the marked enlargement of the joints is not so common. Infected flocks (Snoeyenbos, 1956) usually have low morbidity (1 to 20 per cent) but mortality resulting from cannibalism is significant. Lameness is the most prominent symptom. Warm fluctuating swellings of one or more joints of lame birds are usually found. Occasionally there is an enlargement of the sternal bursa. Severely affected birds lose weight, but many birds less severely affected make satisfactory weight gains when separated from the flock. In experimentally infected turkeys (Olson et al., 1956), the first noticeable sign is failure of the bird to grow (Fig. 28.4). Noticeable swellings in joints are not always present; however, when the hock joints are opened, a small amount of purulent exudate is present. The agent is recovered from this exudate after injection into embryonating eggs, and inoculation of the exudate into chickens produces signs and lesions of synovitis. Further work is needed to determine the incidence of the disease in turkeys.

Treatment in turkeys. Protection is af-

forded by CTC at 200 gm. per ton of feed during prophylactic medication of turkeys. Partial protection is produced by levels as low as 50 gm. per ton. The comparative efficacy in turkeys of the tetra-

cycline antibiotics has not been determined. Treatment of affected birds with streptomycin or CTC has given discouraging results. Prophylactic medication of turkeys has not been economically feasible.

REFERENCES

Badstue, P. B.: 1961. Infectious synovitis. Nord. Vet. Med. 13:561.

Benton, W. J., and Cover, M. S.: 1959. The infectivity of blood and tissues from chickens with infectious synovitis. Avian Dis. 3:561.

Bigland, C. H., and Brown, J. A.: 1955. A suspected case of infectious synovitis in Alberta. Canad. Jour. Comp. Med. 19:251.

Bletner, J. K., Shelton, D. C., Olson, N. O., and Weakley, C. E., Jr.: 1957. Control of infectious synovitis. 3. The efficacy of chlortetracycline with relation to time of experimental infection. Poultry Sci. 36:1016.

Burtscher, H.: 1961. Zum Vorkommen der infektiosen synovitis in Osterreich. Wiener tierärztl Monatschr. 48:850.

Carnaghan, R. B. A.: 1959. An outbreak of infectious synovitis in chickens. Vet. Record 71:81.
——: 1961. The egg transmission of infectious synovitis. Jour. Comp. Path. and Therap. 71:279.

Casorso, R. D., and Junghere, E. L.: 1959. The response of the developing chicken embryo to certain avian pathogens. Am. Jour. Vet. Res. 20:547.

Cassidy, D. R., and Grumbles, L. C.: 1959. Immunity studies on avian infectious synovitis. Avian Dis. 3:126.

Chalquest, R. R.: 1962. Cultivation of the infectious-synovitis-type pleuropneumonia-like organisms. Avian Dis. 6:36.
——, and Fabricant, J.: 1960. Pleuropneumonia-like organisms associated with synovitis in fowls. Avian Dis. 4:515.
——, and Halfhill, J.: 1962. Preparation of antigens and antiserum for the infectious synovitis-type pleuropneumonia-like organisms. Jour. Bact. 84:591.

Cole, D.: 1964. Personal communication.

Cosgrove, A. S.: 1957. Laboratory and field studies with furazolidone in the prevention and treatment of avian infectious synovitis. Jour. Am. Vet. Med. Assn. 130:286.

Cover, M. S., and Benton, W. J.: 1957. The distribution of the infectious synovitis agent in the tissues of artificially infected chickens Avian Dis. 1:312.
——, Benton, W. J., Green, L. M., and D'Armi, F.: 1959. Potentiation of tetracycline antibiotics with terephthalie acid and low dietary calcium. Avian Dis. 3:353.
——, Geletz, J. N., and Waller, E. F.: 1956 The etiology of an arthritic disease of chickens Am Jour. Vet. Res. 17:12.

Domermuth, C. H.: 1962. Experimental production of "breast blisters" by S 6 type Mycoplasma. Avian Dis. 6:135.

Edward, D. G., and Fitzgerald, W. A.: 1954. Inhibition of growth of pleuropneumonia-like organisms by antibody. Jour. Path. and Bact 68:23.

Fabricant, J.: 1960. Serological studies of avian pleuropneumonia-like organisms (PPLO) with Edward's technique. Avian Dis. 4:505.

Guillon, J. C., Renault, L., and Petit, E.: 1962. Quelques aspects de la synovite infectoeuse aviare en France. Rec. Med. Vet. 138:5.

Kerr, K. M., and Olson, N. O.: 1964. Control of infectious synovitis. 14. The effect of age of chickens on the susceptibility of three agents. Avian Dis. 8:256.

Lecce, J. G.: 1960. Porcine polyarteritis and arthritis. Isolation of a fastidious pleuropneumonia organism and Hemophilus influenzae suis. Biology of the pleuropneumonia-like organisms. Annals of the N.Y. Acad. of Sci. 79.670.
——, Sperling, F. G., Hayflick, L., and Stinebring, W.: 1955. Tendovaginitis with arthritis, a new syndrome of chickens. Isolation and characterization of an infective agent. Jour. Exper. Med. 102:489

Munro, D. A., Olson, N. O., Shelton, D. C., Weakley, C. E., Jr.: 1956. Synovitis control. 5. Intramuscular streptomycin and a comparison of continuous and intermittent feeding of Auremycin and Furazolidone (NF-180). Poultry Sci. 35:1161.

Olson, N. O.: 1956. Unpublished data.
——: 1959. Transmissible synovitis of poultry. Laboratory Investigation 8:1384.
——: 1960. Diagnosis of infectious synovitis. Proc. 64th Ann. Meet., Livestock Sanit. Assn. 424.
——, Adler, H. E. DaMassa, A. J., and Corstvet, R. E.: 1964a. The effect of intranasal exposure to Mycoplasma synoviae and infectious bronchitis on development of lesions and agglutinin. Avian Dis. 8:623.
——, Bletner, J. K., Shelton, D. C., Munro, D. A., and Anderson, G. C.: 1954. Enlarged joint condition in poultry caused by an infectious agent. Poultry Sci. 33:1075.
——, Kerr, K. M., and Campbell, A.: 1963. Control of infectious synovitis 12. Preparation of an agglutination test antigen. Avian Dis. 7:310.

———, Kerr, K. M., Campbell, A.: 1964b Control of infectious synovitis. 13. The antigen study of three strains. Avian Dis. 8:209.

———, Munro, D. A., Bletner, J. K., and Shelton, D. C.: 1955. The production of synovitis in chickens by agents from different sources isolated in the yolk sac of embryonating chicken eggs. Poultry Sci. 34:1213. (Abst)

——— and Shelton, D. C.: 1958a. Control of infectious synovitis in chickens. 6. Chlortetracycline in field experiments. Jour. Am. Vet. Med. Assn. 132:477.

——— and Shelton, D. C.: 1958b. Infectious synovitis control. 10. Chlortetracycline in chicks inoculated at one day of age. Proc. U.S. Livestock Sanit. Assn. 62nd Ann. Meet. 201.

——— and Shelton, D. C.: 1959. Infectious synovitis control. 8. Degree of infection and medication. Avian Dis. 3:512.

———, Shelton, D. C., Bletner, J. K., Munro, D. A., and Anderson, G. C.: 1956. Studies of infectious synovitis in chickens. Am. Jour. Vet. Res. 17:747.

———, Shelton, D. C., Bletner, J. K., and Weakley, C. E., Jr.: 1957a. Infectious synovitis control. 2. A comparison of levels of antibiotics. Am. Jour. Vet. Res. 18:200.

———, Shelton, D. C., Munro, D. A.: 1957b. Infectious synovitis control by medication. Effect of strain differences and pleuropneumonia-like organisms. Am. Jour. Vet. Res. 18:755.

———, Shelton, D. C., Munro, D. A., and Bletner, R.: 1957c. Preliminary blood studies in chickens with a synovitis caused by the infectious synovitis agent, pleuropneumonia like organisms and a combination of the two agents. Avian Dis. 1:82

———, Yamamoto, R., Ortmayer, H.: 1965. Antigenic relationship between Mycoplasma synoviae and M. gallisepticum. Am. Jour. Vet. Res., 26:199.

Price, K. E., and Zolli, Z., Jr.: 1959. The influence of terephthalic acid on oxytetracycline serum levels in chickens. Studies on mode of action. J. Avian Dis. 3:157.

Sevoian, M., Snoeyenbos, G. H., Basch, H., and Reynolds, L.: 1957. Studies of infectious synovitis. Avian Dis. 1:121.

———, Snoeyenbos, G. H., Basch, H., and Reynolds, L.: 1958. Infectious synovitis. 1. Clinical and pathological manifestations. Avian Dis. 2:499.

Shelton, D. C., Bletner, J. K., Olson, N. O., Anderson, G. C., and Weakley, C. E., Jr.: 1957. Control of infectious synovitis. 1. Continuous feeding of antibiotics and the influence of diethylstilbestrol and coccidiostats. Poultry Sci. 36:113.

——— and Olson, N. O.: 1957. Infectious synovitis control. 7. Comparison of tetracycline antibiotics. Poultry Sci. 36:1157.

——— and Olson, N. O.: 1958a. Infectious synovitis control. 9. The efficacy of dihydrostreptomycin sulfate as related to time of experimental infection. Antibiotics Annual. P. 272.

——— and Olson, N. O.: 1958b. Control of infectious synovitis. 11. The potentiating effect of terephthalic acid on chlortetracycline. Avian Dis. 2:450.

——— and Olson, N. O.: 1960. Serum proteins of chicks with infectious and mycoplasma synovitis. Poultry Sci. 39:112.

——— and Olson, N. O.: 1961. Effect of terephthalic acid on the activity of chlortetracycline and oxytetracycline. Avian Dis. 5:25.

———, Olson, N. O., and Weakley, C. E., Jr.: 1960. Control of infectious synovitis. 4. Antibiotics and nitrofurans. Poultry Sci. 37:610.

Skamser, L. M., and Seeger, K. C.: 1960. Aureomycin chlortetracycline as a preventive for experimental synovitis. Avian Dis. 4:42.

Snoeyenbos, G. H.: 1958. Infectious synovitis in turkeys. Poultry Pathologist Conference. American Cyanamid Co., Oct. 28.

——— and Basch, H. I.: 1958. A further indication of egg transmission of infectious synovitis. Avian Dis. 2:494.

———, Basch, H. I., and Sevoian, M.: 1958. Infectious synovitis. II. Drug prophylaxis and therapy. Avian Dis. 2:514.

——— and Olesiuk, O. M.: 1955. Studies of an agent producing arthritis in turkeys. Proc. Ann. Pullorum Conf., Univ. New Hampshire, Durham.

Swanson, E. E., Huisman, C. N., Williams, W. L., and Jukes, T. H.: 1956. The effect of calcium levels on aureomycin absorption. Poultry Sci. 35:1251. (Abst.)

Thayer, S. C., Strout, R. G., and Dunlop, W. R.: 1958. Observations on infectious synovitis. Poultry Sci. 37:449.

Wassermann, B., Yates, V. J., and Fry, D. E.: 1953. The cultivation of certain strains of the chronic respiratory disease agent and the turkey sinusitis agent in the inboratarsal joints of chickens. Proc. Ann. Conf. Lab. Workers in Pullorum Dis. Control. Univ. of Mass., Amherst.

Wichmann, R. W., Bankowski, R. A., and DaMassa, A. J.: 1960. The cultivation and modification of the avian infectious synovitis agent in tissue culture. Avian Dis. 4:152.

Wills, F. K.: 1954a. Preliminary report on transmission of an agent producing arthritis in chickens. Texas Agr. Exper. Sta. Prog. Rep. 1674.

———: 1954b. Observations on an agent producing arthritis in chickens. Texas Agr. Exper. Sta. Prog. Rept. 1723.

———: 1955. Study of an unidentified agent producing arthritis in chickens. Southwestern Vet. 8, Winter, No. 2:146.

——— and Delaplane, J. P.: 1955. Transmission and therapy studies on an agent which produces arthritis in chickens. Proc. Book, Am. Vet. Med. Assn. 350.

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29

Rabies, Infectious Equine Anemia and Foot-and-Mouth Disease in Fowl

Rabies in Fowl

Rabies is an acute infectious disease caused by a filterable virus and characterized by symptoms of a central nervous disturbance, progressive paralysis, and followed by death in most animals Gibier (1884) one of the first to carry on experimental studies on rabies infection in fowl, was successful in transmitting the disease to chickens and reinfecting mammals with virus recovered from diseased birds. Spontaneous recovery in experimentally infected birds was recorded.

Kraus and Clairmont (1900) studied the susceptibility of various species of birds to rabies, variations in the course of the disease, and the clinical symptoms observed. The raven, falcon, and old pigeons were refractory to rabies infection. Old pigeons could be infected after a period of starvation. Young pigeons were susceptible to rabies. Considerable variation in the incubation period — from 2 weeks in owls and geese to 40 days or more in chickens — was recorded. Gradual recovery in some birds was reported. Vaccines made from avian tissues were ineffective for immunization. Rabies was transmitted from birds to rabbits, and the incubation period was found to be progressively extended until the virus was rendered inactive. Incoordination and paresis, followed by paralysis and death, were the clinical manifestations observed. Lesions in the brain and spinal cord were similar to those found in man and animals.

V. Löte (1904) found that some birds of prey were susceptible to rabies. He infected a mouse hawk (*Buteo vulgaris*) subdurally with virus secured from the brain of a rabbit. Symptoms of a central nervous disturbance and loss of appetite were recorded 11 days following inoculation. Convulsions of short duration were also observed. The bird could no longer stand, and it lay on its right side pros-

trated 3 days after the first appearance of clinical manifestations. The bird was dead the following day. V. Löte transmitted rabies to two eagle owls which died 2½ and 9 months, respectively, after inoculation without developing any appreciable clinical symptoms. He transmitted rabies to guinea pigs with brain tissue from these birds. Chickens and pigeons were considered to be more resistant to artificial infection than birds of prey. Only one of three cocks experimentally infected with rabies virus contracted the disease. The course of the disease was unusual. The first symptoms developed after a 43-day incubation period and included evidence of incoordination and refusal of food. Definite improvement was noted 3 days after the first appearance of clinical symptoms. The subject appeared to be normal for 14 days but then developed severe paralytic symptoms. One week later the bird had made a complete recovery. This disease ran a similar course in an experimentally infected hen which recovered completely.

Marie (1904) reported a great variation in the incubation period and course of rabies in birds. He attempted to increase the neutralizing power of the sera of mature pigeons immune to rabies by hyperimmunization; this was unsuccessful. Rabies "street" virus, passed through birds repeatedly, gradually decreased in virulence to an inactivated state in which it was no longer capable of producing any reaction in mammals. Active virus, after seven or more serial passages through birds, was so altered that suitable quantities of brain emulsion from these infected birds, injected intraperitoneally or subcutaneously, protected mammals against intraocular inoculation of "street" virus.

Remlinger and Bailly (1936) transmitted rabies to the stork (Ciconia ciconia) by intracerebral inoculation of "street" virus. The symptoms manifested were exclusively of the paralytic type. The experimental transmission of rabies to the pheasant (Diardigallus diardi B.P.) was reported by Jacotot (1938).

The occurrence of rabies in the chicken under natural conditions was considered comparatively rare by Remlinger and Bailly (1929a, b). They successfully transmitted the disease to the bird by bites inflicted on the comb by a rabid dog. Manifestations of the furious type or the paralytic form may develop after a long or short incubation period. The diseased bird may attack its mates or other animals in the furious form of the disease and can be considered a potential source of danger in transmitting the disease to animals and man.

Very little definite proof of the occurrence of spontaneous rabies in fowl can be found in published reports. Experimental studies have provided practically all of our information regarding the avian form of this disease. Schweinburg (1928) reported a case in which a patient had been injured by a rabid hen. The hen showed symptoms of the furious form of rabies for a period of 3 days.

REFERENCES

Gibier, P.: 1884. Recherches expérimentales sur la rage. Abst. Compt. rend. Acad Sci. 98:531.
Jacotot, H.: 1938. Transmission de la rage au faisan (Diardigallus diardi B.P.). Compt. rend. Soc. de biol. 127:131.
Kraus, R., and Clairmont, P.: 1900. Über experimentelle Lyssa bei Vögeln. Zeitschr. f. Hyg. 33:1.
Marie, M. A.: 1904. Note sur la rage chez les oiseaux. Compt. rend. Soc. de biol. 56.573.
Remlinger, P., and Bailly, J.: 1929a. La rage du coq. Ann. Inst. Past. 43-153.
——, and Bailly, J.: 1929b. Nouvelles observations relatives à la rage du coq. Bul. de l'Académie Vét. 82:286.
——, and Bailly, J.: 1936. Transmission de la rage à la cigogne (Ciconia ciconia). Compt. rend. Soc. de biol. 123:583.
Schweinburg, F.: 1928. Seuchenbekämpfung. Jahresber. vet. Med. 48.930.
v. Löte, H.: 1904. Beiträge zur Kenntnis der experimentellen Lyssa der Vögel. Zentralbl. f. Bakt. I. Orig. 35.741.

Infectious Equine Anemia in Fowl

Infectious equine anemia, or swamp fever, is caused by a filterable virus. The transmissibility of this disease to birds is a controversial question, and the evidence in support of this assumption is not adequate and lacks confirmation. Reports found in the literature are of interest and should be reviewed. Oppermann and Lauterbach (1928) claimed that chickens may be infected with the virus of infectious anemia of horses. The histopathologic changes in the liver of birds were considered to be similar to those found in diseased horses. Round cell infiltration and hemosiderin deposits in the liver were thought to be significant. They further claimed that the disease could be diagnosed in horses on farms where significant liver changes associated with infectious equine anemia were found in chickens. Chickens were reported to have been infected with manure of horses suffering from infectious anemia, producing typical liver changes. No specific clinical symptoms could be produced in birds, but Oppermann and Lauterbach concluded that the disease assumed a very mild form and resulted in a significant reduction in the number of red blood cells. They thought it probable that the hemosiderin deposition in the liver resulted from the destruction of the erythrocytes. Certain death losses in chickens were attributed to this type of infection. The liver changes in the chicken which Oppermann and Lauterbach considered characteristic of

infectious equine anemia were found most consistently in chickens killed 5 to 7 days after infection. They also believed that since man is also susceptible to this virus infection, spontaneous infections of birds are factors in the control of the disease.

Balozet (1937) could not transmit infectious equine anemia to chickens nor was he able to recover the virus from inoculated birds. He reported birds to be completely refractory to the disease. Gochenour et al. (1938) concluded, after years of experimental investigations, that this disease was confined largely to horses, mules, and asses. A few cases had been reported in man. Stein (1940) found no alterations in the appearance, condition, or development of chicken embryos following inoculation of the chorio-allantoic sac of 5- to 12-day-old embryos. Furthermore, there was no evidence of unfavorable reactions on the development of any of the embryos, following the intravenous inoculation of virulent blood taken from horses during a febrile attack, when 11-day-old chicken embryos were used.

No conclusive evidence has been found, in spite of the extensive investigations conducted in recent years, to indicate that this disease may be transmitted to birds. The failure to transmit the disease from inoculated birds to the horse, mule, or ass and the inability to produce clinical manifestations in inoculated birds seem to indicate that birds are completely refractory to the disease.

REFERENCES

Balozet, L.: 1937. Etudes expérimentales sur l'anémie infectieuse des équidés. Arch. Inst. Past. de Tunis 26 27.

Gochenour, W. S., Stein, C. D., and Osteen, O. L.: 1938. Infectious anemia. U.S.D.A. Farmers' Bul. No. 1819.

Oppermann and Lauterbach: 1928 Die Diagnose der infektiosen Anämie des Pferdes mit Hilfe des Hühnererkrankes. Deutsch. tierärzl Wochenschr, 36: (Festschrift alter p. 878) 61.

Stein, C. D.: 1910. Report of the Chief of the Bureau of Animal Industry.

Foot-and-Mouth Disease in Fowl*

The early literature includes references to observations made by Wildner, Spinola,

and Becker on the clinical occurrence of foot-and-mouth disease in fowl. References to these reports can be found in Hutyra and Marek (1905), Ehrhardt (1914), and van Heelsbergen (1929). The con-

ditions described indicated both local and general reactions. Small vesicular lesions on the comb, the conjunctiva, in the region of the nostrils, wattles, mouth, throat, and toes were observed. Eroded areas, which developed several days after the rupturing of the vesicles, healed over in a week or two. Following significant temperature reactions, a weakness and depression occurred due in part to the inability of the birds to eat because of the developing lesions in the mouth and throat. The course of the disease was 1 or 2 weeks, followed by complete recovery. Uncomplicated cases were not considered fatal. The early clinical diagnoses which were not confirmed by experimental investigations cannot be considered as definite proof that these conditions were foot-and-mouth disease. According to van Heelsbergen (1929), the lesions described by the early investigators resembled those of fowl pox. He also referred to a vesicular "eczema" on the comb and wattles of chickens similar in appearance to the lesions of fowl pox. Furthermore, these conditions were reported in areas which were definitely free from foot-and-mouth disease. The relationship between these various vesicular manifestations in fowl and foot-and-mouth disease still has to be determined, according to Reis and Nobrega (1936).

The transmission of foot-and-mouth disease to fowl by experimental feeding or inoculation was unsuccessful as reported by van Heelsbergen (1929). Three of twelve fowl fed large amounts of guinea pig virus passed active virus in their feces between 10 and 24 hours but not later than 26 hours, as detected by experimental calf inoculation tests reported by Minett (1927). Further reports indicate the inability to transmit the disease to sea gulls, ducks, sparrows, and martins. Galloway (1937) reported that thirteen out of sixteen adult wild ducks inoculated intradermally into the pads at the base of the feet and digits showed vesicles on the upper surfaces of the web 2 or 3 days after inoculation. The disease was transmitted through eight consecutive duck

passages by the inoculation of virus secured from the vesicles on the feet.

There has been considerable speculation for a number of years on the possible spread of foot-and-mouth disease by birds. Extensive research by British scientists indicates that some degree of susceptibility of birds to infection may exist, but there is little evidence to support such a hypothesis. There is a remote possibility that sea gulls and other fowl which feed on farm land at times and travel great distances might act as mechanical carriers of the virus of foot-and-mouth disease. The experiences and observations of various authorities in this country have never indicated that fowl ever become reservoirs or vectors of the foot-and-mouth virus. Man has been considered as one of the more important factors in the mechanical spread of this virus disease by such authorities as Kling and Höjer (1926), Waßmann and Hirschfelder (1938), and Kling et al. (1939).

The propagation of foot-and-mouth disease virus in the chorio-allantoic membrane of the embryonated eggs of the hen and duck were unsuccessful as reported by Galloway in 1937. Only occasional passage of the virus from one embryonated duck egg to another was experienced, but further passages were unsuccessful. This could possibly have been due to virus dilution, as no evidence of the multiplication of the virus could be detected. Twenty serial passages of virus in chicken embryos were reported by Peragallo (1937), but Richter (1939) was unable to confirm this work. Traub and Schneider (1948) were able to cultivate a strain of foot-and-mouth disease virus of the Vallée O type in chicken embryos. Hecke (1932) propagated foot-and-mouth disease virus in tissue cultures containing epithelial tissues of embryo guinea pigs. This virus may be grown in tissue cultures containing embryonic tissues from species which are susceptible to the disease.

Skinner (1954) infected baby chicks a few hours old with Vallée O type of foot-and-mouth disease virus of cattle origin after the 85th mouse passage. The virus

from infected chicks was obtained from the muscles and hearts 4 days after inoculation and injected into mice 1 week of age. During 12 alternate passages of the virus in chicks and mice, virus from the blood of chicks was recovered over a period of 3 to 5 days after inoculation. Other chicken tissues were tested for virus after macroscopic tongue lesions were observed subsequent to the 8th alternate passage. Virus was found in tongue tissue. Later, using the original cattle strain for intramuscular inoculation into newly hatched chicks, well developed tongue lesions were observed 1 or 2 days later. The lesions often involved the dorsal epithelial surface and were similar to characteristic lesions usually observed in other susceptible species. Histological examinations confirmed the clinical observations. Local lesions developed following direct inoculation of preparations made from tongue epithelium into older birds. Six serial passages of a cattle strain were made following direct inoculation of preparations in birds 2 to 4 months old.

Intramuscular inoculation of large doses of Vallée O type virus of cattle origin and three stock guinea pig strains O, A, and C types, in newly hatched chicks, subsequently produced tongue lesions. The guinea pig strains were successfully passaged four to six times by intradermal inoculation of the tongue of older birds. Two of these strains produced small areas of separation of the lower epithelial structures on the under surface of the feet of newly hatched chicks when inoculated intramuscularly or by direct inoculation in the region of the central foot pad. The development of light local lesions was followed by definite secondary lesions on the tongue. There was no severe systemic reaction during the course of infection in the experimental chickens of all ages. Epithelial lesions completely exfoliated in 1 or 2 days, and no scars or blemishes were left on the tongue.

Virus from selected material was successfully transmitted to 14-day-old chicken embryos. The myocardium seemed to be the main area of virus multiplication in this type of embryo. The highest concentration of virus was secured after 5 or 6 days' incubation at 35° C. The susceptibility of the embryos to infection was reduced considerably by incubation at higher temperatures. Infection was usually followed by the death of the inoculated embryo in 3 to 6 days using inoculum conditioned by eight serial passages or more. Distinct cardiac lesions could be found upon examination.

Failure of the earlier workers to propagate foot-and-mouth disease virus in chicken embryos using the chorio-allantoic method of inoculation has been generally accepted. Success has been reported only in a few instances. The more recent technique, using the intravenous method of inoculation and the subsequent incubation at 35° C. for at least 3 hours, may be the vital factors concerned in successful propagation of foot-and-mouth disease virus in the embryonated chicken egg.

The following diagnostic procedure, as formulated by Olitsky and Schoening in a previous edition of *Diseases of Poultry*, is still valid. "The diagnosis of a virus recovered from fowl, such as that of foot-and-mouth disease, is made by the cutaneous injection of vesicular fluid in the scarified foot pads of healthy, adult guinea pigs or on tongues of normal, previously unexposed cattle. Twelve hours to 5 days later characteristic vesicles appear which are transmissible in series to normal guinea pigs. There are at present three distinct types of virus; according to German (Waldmann) classification they are A, B, and C; according to the French (Vallée) terminology, the A is called O and the B is called A, the type C being designated in the same way as in the German classification. A number of immunologic variants have been identified in both type O and A viruses. The type of virus as well as the character of variants is determined by cross-immunity tests in guinea pigs or cattle, as well as by complement-fixation and neutralization tests. Type specific antisera for these tests are ordi-

narily derived from hyperimmunized guinea pigs."[1]

[1] It should be stressed at this point that the importation of foot-and-mouth disease virus into the United States is prohibited by national statute (Public Law 496, 80th Congress, approved April 24, 1948).

The only research program on foot-and-mouth disease in this country is being carried on by the federal government under strict supervision at Plum Island.

REFERENCES

Ehrhardt, H. W.: 1914. Die Krankheiten des Hausgeflügels. Aarau E. Wirz. Third Ed. Quoted by Ward, A. R., and Gallagher, B. A. 1926. Diseases of Domesticated Birds. The Macmillan Co., New York. P. 142.

Frenkel, H. S.: 1951. Research on foot-and-mouth disease. III. The cultivation of the virus on a practical scale in explantations of bovine tongue epithelium. Am. Jour. Vet. Res. 12:187.

Galloway, I. A.: 1937. Fifth Progress Report of the Foot-and-Mouth Disease Research Committee, H. M. Stationery Office, London. Pp. 29, 364, 369.

Hecke, F.: 1932. Die Eignung verschiedener Gewebsarten zur Züchtung des Maul- und Klauenseuchevirus. Zentralbl. f. Bakt. I. Orig. 125:321.

Hutyra, F., and Marek, J.: 1905. Spezielle Pathologie und Therapie der Haustiere. Gustav Fischer, Jena. I:307–8.

Kling, C., and Hojer, A.: 1926. Recherches sur le mode de propagation de la fièvre aphteuse. Transmission du contage. Compt. rend. Soc. de biol. 94:615.

———, Huss, R., and Olin, G.: 1939. Présence du virus de la fièvre aphteuse dans le contenu intestinal d'un sujet humain vivant dans un milieu infecté. Compt. rend. Soc. de biol. 131:478.

Minett, F. C.: 1927. Second Progress Report of the Foot-and-Mouth Disease Research Committee, H. M. Stationery Office, London. Pp. 18, 34, 50.

Peragallo, I.: 1937. Untersuchungen über das Aphthenvirus I. Mitteilung: Gedeihen des Aphthenseuchevirus auf der Chorioallantois von Hühnerembryonen und seine serienweise Übertragung. Zentralbl. f. Bakt. I. Orig. 140:116.

Reis, J., and Nobrega, P.: 1936. Tratado de Doencas das Aves. Inst. Biol., São Paulo. P. 45.

Richter, H. A.: 1939. Ist das Maul- und Klauenseuchevirus auf dem Allanto-Chorion von Hühnerembryonen nach der Methode von Peragallo zuchtbar? Zentralbl. f. Bakt. I. Orig. 143.273.

Skinner, H. H.: 1954. Infection of chickens and chick embryos with the viruses of foot-and-mouth disease and vesicular stomatitis. Nature 174:1052.

Traub, E., and Schneider, B.: 1948. Zuchtung des Virus der Maul- und Klauenseuche im brüteten Hühnerei. Zeitschr. f. Naturforschung. 3b:178.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart. Pp. 508–10.

Waldmann, O., and Hirschfelder, H.: 1938. Die epizootische Bedeutung der Ratten, des Wildes, der Vögel und der Insekten für die Verbreitung der Maul- und Klauenseuche. Berliner tierarzt. Wochenschr. 54:229.

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30

Duck Virus Hepatitis

A new and highly fatal disease of young White Pekin ducklings broke out during the spring of 1949 in the duck-raising section of Long Island, New York (Levine and Fabricant, 1950). The disease spread rapidly, and before the summer was over practically all of the seventy-odd duck farms in the area had suffered losses. At first, ducks 2 to 3 weeks of age were affected. Gradually the disease attacked younger birds until ducklings less than a week old were succumbing. On severely affected farms, mortalities up to 95 per cent were not uncommon in some broods. Successive lots of ducks almost invariably became infected. Later, occasional broods would escape with little mortality. It was estimated that 15 per cent of the total number of ducklings started for that year died from the disease, a total of 750,000 birds. In the United States the disease has been diagnosed in Massachusetts, Illinois (Hanson and Alberts, 1956), and Michigan. It has also been reported from Canada

(Macpherson and Avery, 1957), England (Asplin and McLauchlan, 1954; Asplin, 1956), Germany and Egypt (Shehata and Reuss, 1957), the Netherlands (Smits, 1957), Belgium (Schyns, 1957), Italy (Rossi and Pina, 1957; Agrimi, 1958), Russia (Prokofiva and Doroshko, 1960), and Hungary (Derzsy cited by Reuss 1959a).

Signs. The onset and spread of the disease was very rapid, with practically all the mortality occurring within 3 or 4 days. Affected ducklings at first failed to keep up with the brood. Within a short time the birds stopped moving, and squatted down with eyes partially closed. The ducklings fell on their sides, kicked spasmodically with both legs, and died with heads drawn back (Fig. 30.1). Death occurred within an hour or so after signs were noted. During the height of severe outbreaks, the rapidity with which ducklings died was astonishing.

Gross lesions. The principal damage was found in the liver. It was enlarged and
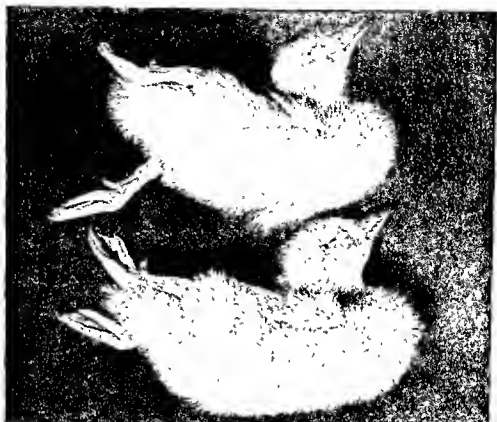
FIG. 30.1 – Ducklings dead from infection with virus hepatitis. Note typical opisthotonus.
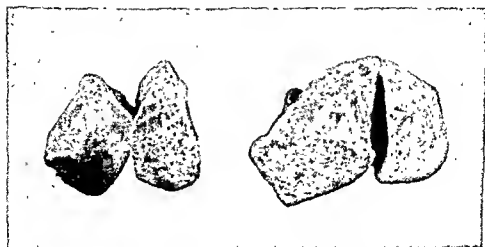
FIG. 30.2 – Livers with hemorrhagic lesions caused by duck virus hepatitis infection.

contained punctate or ecchymotic hemorrhages (Fig. 30.2). Frequent reddish discoloration or mottling of the liver surface was seen. The spleen was sometimes enlarged and mottled. In numerous cases the kidneys were swollen and the renal blood vessels injected. These lesions were reproduced in young ducks by inoculation and feeding of egg-propagated virus.

The older ducks examined at the first appearance of the disease had a marked pericarditis and air sac infection characterized by a whitish-yellow, fibrinous deposit. It is now believed that these lesions probably were produced by another condition and were not related to the virus hepatitis infection even though that virus could be isolated. As the younger ducklings became affected, the typical liver lesions made their appearance.

The microscopic changes in uncomplicated, experimentally induced infections have been studied (Fabricant et al., 1957). The primary changes consisted of necrosis of the hepatic cells and proliferation of the bile duct epithelium. Varying degrees of inflammatory cell response and hemorrhage occurred. Regeneration of the liver parenchyma was observed in ducklings that did not die.

Host specificity. In the field, the disease only occurred in young ducklings. Adult breeders on infected premises did not become infected. These birds continued in full production, and their eggs were highly fertile with excellent hatchability. One attempt to infect breeders with egg-propagated virus failed. No experiments were made to attempt transmission of this disease to other species of birds. Field observations indicated that chickens were not susceptible, since broiler chicks being brooded in the same pens where ducklings were dying failed to become infected. On one farm, turkeys being reared on a slatted platform attached to the side of a duck brooder house where the disease was present were not affected.

Schoop et al., (1959) and Reuss (1959a) failed to infect chickens experimentally. The latter worker could not transmit the disease to rabbits, guinea pigs, white mice, or dogs.

Etiology. No bacterial agent could be isolated from the sick ducklings. Infected material treated with penicillin and streptomycin and inoculated into the allantoic sac of 9 day-old chicken embryos yielded a virus. On subsequent study the virus proved to be one that hitherto had been undescribed. Practically all tissues, including the blood, yielded this infective agent. Embryos that either died on the fifth or sixth day or were destroyed on the sixth day were stunted and edematous (Fig. 30.3). The edema was noted especially around the thigh and abdomen. The amnionic sac contained an excess of fluid. The yolk sac was reduced in size and the contents were more viscous than usual. A greenish discoloration of the embryonic fluid and yolk sac was often found and could be detected on candling of the eggs. The livers of the embryos often were greenish in color and frequently had whitish-yellow, necrotic foci, varying from pinhead in size to larger areas involving considerable portions of the parenchyma. Allantoic fluids from these embryos and from infected duck tissues contained the virus and killed from 10 to 60 per cent of the embryos by the sixth day.

The virus could be passed through both the Seitz and Berkefeld W filters, as evidenced by the production of typical embryo lesions with filtrates. The hepatitis virus did not agglutinate chicken red cells nor was it neutralized by Newcastle disease antiserum. Through the courtesy of Dr. Osteen of the United States Bureau of Animal Industry, antiserum from the duck plague infection in Holland (Jansen and Kunst, 1949) was obtained. Neutralization of the hepatitis virus with duck plague antiserum did not occur (Fabricant, 1950). Virus neutralization tests with convalescent sera from human and canine virus hepatitis failed to demonstrate any serological relationship of these diseases to duck virus hepatitis (Fabricant et al., 1957).

Reuss (1959a) determined by electron
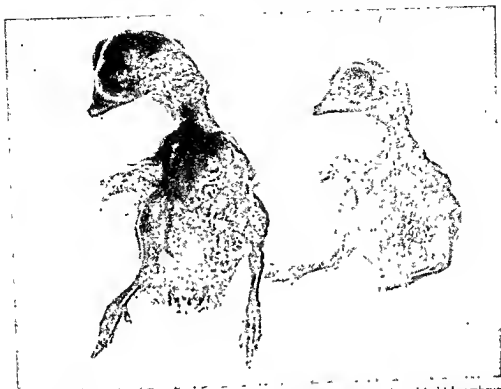
FIG. 30.3 — (Left) A normal 15-day-old chick embryo. (Right) A 15-day-old chick embryo inoculated with duck virus 6 days previously. Note the small size and the edema, especially around the thigh and abdomen. (Courtesy of the Cornell Veterinarian.)

microscopy that the virus was a rounded or spherical particle that measured 20–40 mμ. He was unable to find elementary bodies in tissues nor could he agglutinate chicken red cells with the virus.

The virus survived 8 months at −30° C. (Schyns 1957) and a month at −20° C. but was dead in 6 days at refrigerator temperature and in 4 days at room temperature (Reuss 1959a).

It is interesting to note that early in the investigation Newcastle disease virus was isolated twice from ducks on a farm where losses were occurring. Efforts to incriminate Newcastle disease with the losses failed.

Transmission. Although the high mortality and rapid spread of the disease on farms indicated extreme contagiousness, occasional exceptions were observed. In one pen 65 per cent of the ducks died, while in an adjoining pen separated only by a 14-inch curb, the mortality was negligible.

The first efforts to transmit the disease to small groups of three or four caged ducklings by injection and feeding of egg-propagated virus were not successful. In another experiment, with tissues from a natural outbreak, some of the ducklings became infected. Transmission was most easily accomplished by intramuscular injection and by feeding egg-propagated virus and infected organs to larger groups of ducklings (ten to twenty) kept on litter under a hover. The incubation period was 24 hours in most experiments, and practically all of the deaths took place by the fourth day. Uninoculated ducklings placed in the same pens with the inoculated birds contracted the disease and died somewhat later than the injected ducks.

Egg transmission presumably does not take place. Newly hatched ducklings pro-

duced by breeders on infected premises remained well when taken to premises where no ducks were being kept. Asplin (1958) confirmed this finding.

Diagnosis. The sudden onset, rapid spread, and acute course of this disease is characteristic. Lesions in the livers of young ducklings up to 3 weeks of age are practically pathognomonic. Absence of bacteria and isolation of the virus in chicken embryos, with production of the characteristic lesions previously described, serve further to identify the causative agent. The specific neutralization of the virus by immune duck virus-hepatitis serum is a positive means of identification.

The use of the agar gel diffusion precipitin technique for the identification of the virus was described by Murty and Hanson (1961). There is the possibility of adapting this test for diagnostic purposes.

Prevention and control. The epizootiology of duck virus hepatitis has not been completely worked out. Although numerous possibilities for spread of the disease by visitors, garbage and dead-bird collectors, etc. existed, outbreaks occurred on farms under excellent management and good sanitation.

Unsuccessful attempts were made to immunize ducklings with killed (formalinized) and live virus vaccines made from chicken embryo fluids. It was not until prophylaxis with duck virus-hepatitis antiserum was attempted in controlled experiments that protection from the experimentally transmitted disease was obtained. The subsequent application of serum therapy in the field has proven to be highly successful. Blood from exposed and recovered ducks is collected at the slaughter house and processed. An antiserum bank sufficient to treat four million ducklings is kept on hand at the Duck Disease Laboratory at Eastport, Long Island, under the direction of Dr. William D. Urban (Dougherty, 1953). One-half ml. duck virus-hepatitis antiserum (0.5 per cent phenol added) is injected intramuscularly into all ducklings of a brood when the first

few deaths from the disease occur. The mortality is negligible in the treated ducklings. If, however, a portion of the brood is not treated, the usual heavy mortality occurs. No relapse in treated broods occurred during the summer of 1950. In the past few years, relapses in serum treated broods have necessitated repeated serum injections before losses could be stopped.

Another possible approach to the control problem was described by Asplin (1956), who immunized breeder ducks with hepatitis virus. The parental immunity the dams conferred on their progeny successfully protected the ducklings on challenge with virulent virus. Ducklings produced by nonimmune dams succumbed to challenge. On the other hand, Reuss (1959b) found that ducklings with parental immunity resisted challenge with virulent virus successfully only when their dams had received previously 6 live virus injections. Ducklings from dams injected 3 times were susceptible. Recently, Hwang et al. (1962) have applied the dam immunization procedure for the control of virus hepatitis in ducklings with good success.

Reduction of the pathogenicity of the duck hepatitis virus by serial passage in chick embryos has been reported by Asplin (1958), Schoop et al. (1959), Reuss (1959b), and Hwang and Dougherty (1962). Asplin found that his attenuated virus applied by stabbing the footpad with needles immunized young ducklings. Field application of this vaccine was effective. Reuss (1959b) also reported successful immunization experiments with his attenuated strain.

The virulence and persistence of the field virus, the level of parental immunity in the ducklings, the age and breed susceptibility of the ducklings, the husbandry practices and environmental influences will be some of the factors that will affect the choice of control programs in different localities.

REFERENCES

Agrimi, P.: 1958. L'epatite virale delle anatre *(Levine e Fabricant 1950)*-Signalazione di un episodio. Identificazione del virus e prove di transmissione sperimentale. Zooprofilassi 13:541.

Asplin, F. D.: 1956. *The production of ducklings resistant to virus hepatitis.* Vet. Record 68:412.

——: 1958. An attenuated strain of duck hepatitis virus. Vet. Record 70:1226

——, and McLauchlan, J. D.: 1954. Duck virus hepatitis. Vet. Record 66:456.

Dougherty, E., III: 1953. Disease problems confronting the duck industry. Proc. Book AVMA 19th Ann. Meet. 359.

Fabricant, J.: 1950. Unpublished data.

——, Rickard, C. G., and Levine, P. P.: 1957. *The pathology of duck virus hepatitis.* Avian Dis. 1:256.

Hanson, L. E., and Alberts, J. O.: 1956. Virus hepatitis in ducklings. Jour. Am. Vet. Med. Assn. 128:37.

Hwang, J., and Dougherty, E., III: 1962. Serial passage of duck hepatitis virus in chicken embryos. Avian Dis. 6:435.

——, Ash, W. J., Dougherty, E., III: 1962. Production of passive immunity against viral hepatitis in White Pekin ducklings. Jour. Am. Vet. Med. Assn. 141:1474.

Jansen, J., and Kunst, H.: 1949. Is duck plague related to Newcastle disease or to fowl plague? Rep. 14th Internat. Vet. Cong. London 1949 (2) p. 363.

Levine, P. P., and Fabricant, J.: 1950. A hitherto-undescribed virus disease of ducks in North America. Cornell Vet. 40:71.

Macpherson, I., W., and Avery, R. J.: 1957. Duck virus hepatitis in Canada. Canad. Jour. Comp. Med. 21:26.

Murty, D. K., and Hanson, L. E.: 1961. A modified microgel diffusion method and its application in the study of the virus of duck hepatitis. Am. Jour. Vet. Res. 22:274.

Prokofiva, M. T., and Doroshko, I. N.: 1960. Virus hepatitis of ducklings. (In Russian). Veterinaria. 37:58.

Reuss, U.: 1959a. Virusbiologische Untersuchungen bei der Entenhepatitis. Zentbl. Vet. Med. 6:209.

——: 1959b. Versuche zur aktiven und passiven Immunisierung bei der Virushepatitis der Entenkuken. Zentbl. Vet. Med. 6:808.

Rossi, C., and Pini, A.: 1957. L'epatite da virus degli anatroccoli. Osservazione e ricerche. Vet. Ital. 8:1175.

Schoop, G., Staub, H. and Erguney, K.: 1959. Uber Virushepatitus der Enten. 5. Mitteilung: Versuche zur Adaptation des Virus und embryonierte Huhnereier. Mh. Tierheik. 11:99.

Schyns, P.: 1957. L'hepatite à virus du caneton. Ann. de Med. Vet. 101:264.

Shehata, H., and Reuss, V.: 1957. Virus Hepatitis der Enten in Deutschland. Deutsche tierärztl. Wochenschr. 64:27.

Smits, W. H.: 1957. Voorlopige Mededeling betreffende een Virusziekte bij Eendenkuikens. Tijdschr. v. Diergeneesk. 82:177.

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31

Avian Monocytosis (So-called Pullet Disease), Infectious Nephrosis and Bluecomb Diseases of Turkeys

AVIAN MONOCYTOSIS

Synonyms. Pullet disease, bluecomb, summer disease, housing disease, unknown disease, new disease, X disease (Beaudette, 1929), XX disease, cholera-like disease (Ryff and Stafseth, 1942), contagious indigestion (Waller *et al.*, 1942), battery nephritis, Bright's disease (Weaver, 1941), Tom Barron's disease, acute toxemia or colibacillosis (Weisner, 1941), hepato-nephrosis (Jungherr and Levine, 1941), avian monocytosis (Jungherr and Matterson, 1944), mud fever of turkeys (Peterson and Hymas, 1951), avian infectious diarrhea (Watanabe *et al*, 1951, quoted by Watanabe, 1952), bluecomb disease of turkeys (Pomeroy and Sieburth, 1953), transmissible enteritis of turkeys (Sieburth and Johnson, 1957), uraemia (Hungerford, 1962), infectious nephritis-nephrosis syndrome (Winterfield and Hitchner, 1962), kidney breakdown disease complex (Cumming, 1963a).

While these diseases share clinical, hematologic, and perhaps therapeutic features, recent studies indicate that they include at least 3 separable entities, namely pullet disease of young chicks and an infectious renal disease of chicks caused by a virus related to that of infectious bronchitis, and bluecomb of turkeys recently recognized as a vibriosis. The principal characteristics of these conditions are discussed separately as far as possible.

Under the term "X disease" Beaudette (1929) briefly described a disorder of adult fowl which usually affected heavy birds in high production and was characterized by cyanosis of the comb and wattles and sudden death; flock mortality was comparatively low. At necropsy affected birds showed congestion of the respiratory tract, liver, ovary, kidneys, and intestine, the latter filled with thick catarrhal material. The heart and the abdominal fat surrounding the gizzard showed small hemorrhages; in one case the liver exhibited evidence of necrosis. The disorder revealed an anatomic resemblance

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to fowl cholera, but culture and transmission studies with unfiltered and filtered materials failed to demonstrate an infectious agent.

A similar condition of chickens and occasionally of turkeys had been observed both in the field and in the laboratory, throughout the northeastern states, but no systematic study had been reported until Jungherr and Levine (1940) attempted a pathologic delineation of the syndrome. Although these authors recognized an acute form similar to X disease, and a subacute form primarily characterized by kidney lesions, they found certain microscopic and chemical features to be common to both forms and regarded them as an entity. On purely symptomatic and gross-pathologic grounds, Bullis (1940) believed the acute and subacute forms, termed by him "pullet" and "blue comb" disease, respectively, to represent different entities. This possibility was likewise considered by Beaudette (1940), who differentiated them and applied the names "X disease" and "new wheat poisoning."

These opinions emphasize the common occurrence, in young laying birds, of an important disorder which has the earmarks of an infectious disease but for which a transmissible etiologic agent has not been demonstrated with certainty. Without recognition of "pullet disease" as a definite condition, certain cases of adult morbidity and mortality could not be diagnosed. Watanabe et al. (1951) described a pullet diseaselike condition of chickens under the term "avian infectious diarrhea." They consider the term "avian monocytosis" unsuitable for pullet disease because monocytosis also occurs in Newcastle disease. Similar objections would hold for any pathologic nomenclature of a disease, e.g., coryza. Although these various conditions share certain features in nomenclature, pathology, and therapeutic response, their etiologic interrelationship is not established.

Occurrence. The statistical data on the geographic distribution of pullet disease are limited. Lack of agreement on the morphologic range of the syndrome retards diagnostic classification. Some reports of the disease have been based on symptomatic evidence alone. However, even if one considers only the acute and most easily recognizable form, the occurrence of pullet disease has been reliably reported, aside from New Jersey (Beaudette, 1929), from most of the northeastern states, Michigan (Weisner, 1941), and California (Hurt, 1941), and Ontario (Weaver, 1941). Verbal reports seem to indicate its presence in North Carolina, Utah, and Missouri (reported by Jungherr, 1945).

Gordon and Blaxland (1945) reported the occurrence in England of a disease in poultry resembling the so-called pullet disease in America. Mochizuki (1951), working at the Government Experimental Station for Animal Hygiene in Tokyo, observed an infectious diarrhea in chickens with clinical, pathologic, and hematologic manifestations strikingly similar to the condition under discussion. That this syndrome represents a clinico-pathologic entity of wide geographic distribution and economic importance is indicated by reports from Italy (Caparrini, 1960), Holland (Maas and Voûte, 1961), East Pakistan (Ali, 1961), West Pakistan (Qureshi, 1955), Bombay (Vaishnar and Parnaik, 1961), and Australia (Lindtner, 1960; Ranby, 1958). The literature has been reviewed by Maas (1960) and Chanteclair (1962).

A Connecticut survey for the years 1931–39 (Jungherr and Levine, 1941) showed that pullet diseaselike conditions occurred in 15 per cent of 1,765 survey cases examined; 72 per cent of the positive cases were classified as uncomplicated. These cases were observed in flocks of birds kept on rations prepared according to a standard New England formula, and on 20 different commercial brands. Various poultry breeds were found to be susceptible, the heavy breeds predominating. The majority of the cases occurred between the ages of 5 and 7 months, that is during early production, but pathologically indistinguishable cases were observed in

chicks 4 weeks old and in 2-year-old layers. The available data placed the major seasonal incidence between June and November, with the peak in August. A continuation of the Connecticut survey for the 4 calendar years 1940 to 1943 showed a similar seasonal distribution of the incidence (Jungherr and Matterson, 1944). During 1943 the attack rate was particularly high, namely 34.5 per cent of 269 specimen consignments of chickens 3 months of age or older (Scott et al., 1944). Since that time, the numerical incidence has decreased markedly, a fact for which no ready explanation has become available.

Cole (1950) gave a valuable account of an outbreak in an experimental flock of 2,850 Single Comb White Leghorn chickens, 9 to 17 weeks of age, which had been bred especially for resistance and susceptibility to avian lymphomatosis, and showed a significant nonparallel strain and family difference in mortality from pullet disease. During the 30-day course of the apparently contagious disease, there was a marked intensification of the clinical and pathologic manifestations of the syndrome. Moultrie et al. (1955) obtained additional evidence for genetic variation in resistance to bluecomb disease among families of birds, a factor which was apparently unrelated to mortality from other causes. Strain differences in susceptibility to renal disorders, of which pullet disease is an example, were also reported by Biely and March (1958) and Hicks (1958).

Whereas the incidence of pullet disease in chickens has decreased in the United States during the past years, according to diagnostic reports, a similar condition has been reported in West Pakistan (Qureshi, 1955) and an increase of the acute type in England (Blaxland, 1957).

Symptomatology. In the typical acute form, a large proportion (average 15 to 21 per cent) of an apparently healthy flock shows a sudden affliction which is characterized by depression, lack of appetite, and whitish or watery diarrhea; occasionally there is constipation. Some birds exhibit distension of the crop with sour-smelling contents, darkening of the head (bluecomb or cyanosis), sunken eyes, shriveled legs, and high fever in the terminal stages. Laying flocks undergo a severe drop in egg production. Maas (1961) made a careful study of the effect on egg quality 3 months after an outbreak and found the average egg weight to be 1.23 grams less than that of comparable controls. Mortality is usually sudden and ranges from 50 per cent to almost zero, with an average of about 5 per cent of the flock. Subacute cases are distinguished by a comparatively low, often spotty incidence and prolonged course. The clinical signs in the flock are less intense; but individual birds, according to Weaver (1941), may show severe prostration, oliguria, convulsive symptoms, and impaired vision.

The primary signs of pullet disease are nonspecific in themselves, but when considered together with the seasonal incidence during early active production, they are highly suggestive of the disorder, if known infectious diseases can be ruled out. Sporadic cases often go unnoticed and are classed among the culls.

The course of the disease in most cases extends over a period of from 1 to 2 weeks, and terminates in a high percentage of apparent recovery, especially if prompt attention is given to the ailing flock. Egg production, however, tends to lag for several weeks, and a partial moult may ensue. After the acute attack has subsided, relapses may occur (Weaver, 1941), which simulate the picture of the subacute form. An unusually prolonged course is often complicated by other factors, especially neoplastic diseases.

Pathology. Pullet disease is characterized morphologically by dehydration, necrosis of the liver (spottiness) and pancreas (chalkiness), hemorrhages on the serous membranes, increased mucus in the intestine, various renal changes, and degenerations in skeletal muscle (fish flesh-like) and ovary (soft or broken follicles). The acute form exhibits congestive phenomena, or liver and muscle lesions,

while renal predominate in the subacute form. Blaxland (1957) has compared the anamnestic and pathologic features of the acute and subacute forms on the assumption of a possible etiologic difference. Different combinations of such organic alterations in either gross or microscopic intensity produce a highly variable pathologic picture, especially as revealed by ordinary necropsy technique. This variability holds true for initial as well as follow-up specimens from the same outbreak, so that microscopic, hematologic, and chemical studies are necessary for complete diagnosis.

Birds affected with avian monocytosis are usually well developed and in good flesh, with a tendency to obesity. The appendages of the head appear congested, as well as the mucous membrane of the nasal passages. The vent feathers are soiled by urinary material. The *skeletal muscles*, especially the breast muscles, appear dehydrated and show capillary injection. In some cases circumscribed pale, often turgent (fish fleshlike) areas are seen, which microscopically represent patches of muscular degeneration: the myofibers are either in a state of granular disintegration separated by interstitial

edema or, more characteristically, show loss of striation, fragmentation, and hyaline swelling, associated with mild polynuclear infiltration and incipient regeneration. In other words, they show the features of Zenker's degeneration, as seen in human typhoid fever and other toxic conditions (Fig. 31.1).

Although the *liver* may appear fatty or congested, an infrequent but most typical alteration in this organ is an evenly spaced studding with round yellowish areas about 1 mm. in diameter, which often have a minute hemorrhagic center. These foci may be few in number and may be associated with subcapsular petechiae. There is ordinarily no evidence of hepatic tumefaction or fibrinous exudation. Microscopically, the areas vary in size and represent typical focal necrosis of no particular zonal orientation (Fig. 31.2); pathogenetically they seem to develop either on the basis of simple coagulative necrosis of hepatic cells, or the accumulation of hyaline material in the Kupffer cells leading to sinusoidal thrombosis. The necrotic foci often undergo secondary polynuclear infiltration and may later be replaced by regenerating liver cells. The rest of the parenchyma shows

FIG. 31.1 – Avian monocytosis. Section of breast muscle showing Zenker's degeneration. ×150.
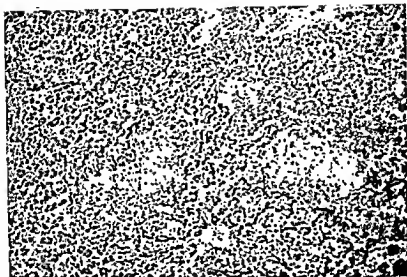
FIG. 31.2 — Avian monocytosis. Section of liver showing focal necrosis. ×150.

FIG. 31.3 — Avian monocytosis. Section of liver. Severe bile stasis. ×150.

marked biliary stasis, especially in the larger ducts (Fig. 31.3).

The *serous surfaces* often reveal multiple but comparatively few and widely spaced punctiform hemorrhages. These tend to occur on the visceral surface of the sternum, on the gizzard and abdominal fat, and on the pericardium. Microscopically, the peritoneal surface of the visceral organs is frequently seen to be covered by a homogeneous eosinophilic material which is infiltrated with heterophils and spherical eosinophilic globules (Fig. 31.4) which are apparently derived from broken egg cells (Jungherr and Levine, 1941).

The *spleen*, as a rule, presents a normal appearance. The lack of tumefaction is helpful in the differential diagnosis and elimination of bacterial and leukotic diseases. Small necrotic foci are observed at times, together with bile- and hemosiderin-laden phagocytes. The *pancreas*, which normally displays a pinkish-gray homogeneous color, is apt to present a chalky appearance which resolves itself into numerous fine whitish areas on close inspection. This change, according to microscopic observation, seems to be brought about principally by cloudy swelling in the center of the acinar lobules, a process which may go on to karyorrhectic necrosis (Jungherr and Matterson, 1944). In addition, the size of the Langerhans' islets appears sometimes increased; their cells are swollen or show here and there pale eosinophilic intranuclear inclusions, which may represent colloidal degeneration products (Jungherr and Levine, 1941). Similar inclusion bodies have since been observed in chickens and turkeys not known to be affected with avian monocytosis by Lucas (1947) and classified as of the multiple homogeneous type, in distinction from the clustered granular type also found in turkeys (Lucas, 1951).

The external surface of the *intestine* is unaltered. The lumen of the ileum is usually filled with turbid tenacious mucus which is often removable as a perfect cast. Histologically the changes are those of catarrhal enteritis. There may be desquamation of the epithelium with the subepithelial zones showing marked increase in cellularity. The inflammatory cells are composed chiefly of mononuclears, lymphocytes, and histiocytes. It is not uncommon to find many cystic crypts con-

FIG. 31.4 — *Avian monocytosis.* Section of pancreas. Serosa shows eosinophilic exudate with globules derived from egg cells. ×150.

FIG. 31.5 — Avian monocytosis. Section of kidney showing a large cast in the center. ×500.

taining inspissated mucus (Jungherr and Matterson, 1914).

The gross lesions of the *kidneys* present a gradient from insignificant changes to marked enlargement, especially of the anterior lobes, and finally the familiar picture of uric nephritis known as visceral gout. Microscopic alterations are frequent in grossly "normal" kidney tissue; they are often of patchy distribution and vary in character. In the most acute cases, one sees extensive cloudy swelling, pyknosis, and desquamation of the epithelium of the proximal convoluted tubuli, a point which can be evaluated only in fresh necropsy material. Other definite renal changes consist of dilatation of tubuli associated with flattening of the epithelium and formation of hyaline casts (Fig. 31.5) and pseudogiant cells (Fig. 31.6) from infolding epithelium. The larger of these foci may show crystalloid radiating centers considered to be pathognomonic for uric nephritis (Siller, 1959). In protracted cases the tubuli show many cellular casts composed of disintegrating heterophils. The glomeruli likewise may exhibit significant alterations in avian monocytosis, such as thickening of the basement membrane, protein precipitate in Bowman's space (Fig. 31.7), adhesions, and dilatation (loculation) or hyaline thrombosis (Weaver, 1941) of the tuft capillaries. Fibrous obliteration of the glomeruli does occur in some instances.

The *ovary*, often being in full production, quite commonly presents irregular soft or broken egg follicles. The yolk material is of normal consistency. Massive fibrinous exudate around the follicles is present, and if present is probably due to secondary bacterial changes.

The similarities in the pathologic concept of avian monocytosis as presented here, and those of Mochizuki et al. (1952), Qureshi (1955), and Blaxland (1957) for the disease of chickens are striking.

Hematology. The blood may show severe hemoconcentration, increased viscosity and coagulability, and low venous pressure. For these reasons it is sometimes difficult to obtain good blood samples by venepuncture. Birds are apt to die in the process. The hemoconcentration is reflected in increased hemoglobin values averaging 15.1 grams per cent in severely affected birds (Jungherr and Matterson, 1944).
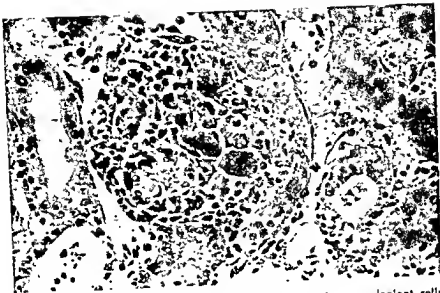
FIG. 31.6 – Avian monocytosis. Section of kidney showing pseudogiant cells in tubuli. ×500.
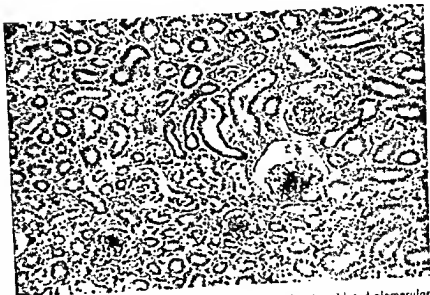
FIG. 31.7 – Avian monocytosis. Section of kidney showing dilated glomerular space containing desquamated cells. ×150.

In hematologic studies by the above authors, there was a consistent but moderate leukocytosis, averaging 40,000 per mm.³ The most significant change in the blood picture was a relative and absolute monocytosis which averaged about 20 per cent or 8,000 per mm.³, respectively, in comparison with the normal of 8.9 per cent for females, or 1,700, according to Olson (1959). The intensity of the blood changes varied with the clinico-pathologic picture and seemed to be particularly marked in cases of kidney involvement. As a rule, the majority of the birds in a specimen consignment showed the monocytic shift which sometimes constituted the only morphologic evidence of the disease. In stained smears the monocytes were ordinarily of the large, mature type but were sometimes characterized by basophilic cytoplasm and rounded nuclei suggestive of immaturity. Mitotic figures were rare. Differential counts by Mochizuki (1951) on 19 birds grouped according to severity of symptoms also showed, in all but one bird, a relative monocytosis ranging from 12 to 52 per cent. Similar results were reported for naturally and artificially infected chickens by Watanabe et al (1951).

The significance of the hematologic findings increased with the certainty with which other diseases such as fowl typhoid and fowl cholera could be ruled out. Since the blood changes seemed to represent the outstanding common denominator in pullet disease cases of varying intensity, the scientific term "avian monocytosis" was proposed (Jungherr and Matterson, 1944).

Chemical pathology. Clinical resemblance of avian monocytosis to uremia and the pathologic evidence of kidney and liver involvement emphasize the importance of the chemico-pathologic aspects. The earlier studies (Jungherr and Levine, 1940) have been extended by Levine and Jungherr (1941). It appears that the blood of birds affected with pullet diseaselike conditions shows high average values for nonprotein nitrogen

(26.8 mgm. per cent), and especially for uric acid (18.9), approximately normal values for phosphorus (5.5), magnesium (2.36), and total ketone bodies (15.5) and usually low values for calcium (13.9 mgm. per cent). The average value for glucose (200) is somewhat high, but wide variations are encountered in both affected and normal birds. In severe cases of avian monocytosis, the values for serum potassium are slightly below normal while those for whole blood potassium are high. Total chlorides may be strikingly low (Jungherr and Matterson, 1944). This chemico-pathologic picture is in keeping with a uremic concept of pullet disease. Very acute cases of avian monocytosis usually fail to show high values for uric acid, but the nonprotein nitrogen may be increased.

Urine analysis of birds with high blood uric acid usually reveals a polycy tosuria; this may be due to hyperglycemia, defective reabsorption on the part of damaged renal tubuli, or both. A reducing substance in birds suffering from experimental nephritis was first observed by Dworin et al. (1941) in this laboratory, and confirmed and identified in field cases of pullet disease by Levine and Jungherr (1941). Albuminuria was frequently observed in laying birds when the urine was obtained by the cloacal technique of Davis (1927) and Coulson and Hughes (1931), while urine obtained by cannulization of exteriorized ureters (Hester et al., 1939–40) was free from albumin. Thus, the albumin seemed to be due to admixture of secretions from the genital tract, and to be of no pathologic significance.

Differential diagnosis. Some phases of the clinical and to a certain extent the pathologic picture of this disorder can be brought about by any of the common infectious diseases such as fowl cholera, pullorum disease, and fowl typhoid. For this reason it is of diagnostic significance to exhaust the possibility of known specific infections. Flock outbreaks can often be suspected from the anamnestic data.

Etiology. The exact factors involved in

the causation of the field syndrome of avian monocytosis are unknown. Etiologic studies have been concerned with the possibility of nephrotoxic substances particularly in wheat, physical factors such as overheating and dehydration, and of infectious agents.

Severe cases of avian monocytosis represent essentially a uremic condition referable to renal damage. Pathologic involvement of the liver in renal diseases is recognized in man under the term "liver death" and/or hepatorenal syndrome (Wilensky, 1939). This would be plausible in birds on account of the close circulatory connection between the two organs and the existence of a special portal circulation in the avian kidney (Spanner, 1925; Gordeuk and Grundy, 1950). Experimental nephrotoxicoses may shed some light on the problem, especially since the demonstration of nephrotoxic antagonists to essential nutrients (ethionine against methionine in rats) by Wachstein and Meisel (1951). In birds, repeated intramuscular injections of potassium dichromate (0.001 per cent of body weight) caused uric nephritis together with occasional necrobiosis of liver, pancreas, and skeletal muscle (Jungherr and Levine, 1941). Feeding chicks certain inorganic acids, particularly sodium citrate and acetate, produced the so-called "salt effect" which was preventable by potassium salts (Correll, 1941). In confirmation of this work, Scott et al. (1944) found the salt effect to be indistinguishable from visceral gout or uric nephritis and to be preventable by potassium-rich molasses and potassium chloride. The latter substance also seemed to have a certain curative effect on spontaneous avian monocytosis. The possible effect of the sodium-potassium balance on the development of "uraemia" was studied by Beilharz and McDonald (1960). In 6 groups of 4-week-old chicks, fed diets varying in protein source and supplementary sodium and potassium chlorides for 8 weeks, an outbreak of uremia was less severe in the potassium supplemented than in the nonsupple-

mented groups. The possibility of a coexisting infectious agent was not ruled out. In man, renal and cardiac lesions associated with chronic diarrhea have been termed "kaliopenic nephropathy," an entity recently studied under the light and electron microscope by Biava et al. (1963). The principal renal lesion was an expansion of normally existing extracellular spaces together with changes in the tubular basement membrane and was considered similar to that in hypernatremia. Selye (1942) produced nephrosclerosis in chicks by repeated subcutaneous injection of desoxycorticosterone acetate, by watering with physiologic salt solutions, or both (Selye and Stone, 1943), and believed the experimental condition to resemble avian monocytosis. Hormonal adrenal deficiency and faulty adaptation have been suggested by Van Ness (1951b) as underlying causes of this disorder; this might find some support from the reported therapeutic response, especially of the subacute form, to antihistamines (0.1 gram Anthisan per os) (Thompson, 1951). High protein diets alone, although producing articular gout in turkeys (Bollman and Schlotthauer, 1936a, b) and in chickens (Oppenheimer, 1941; Oppenheimer and Kunkel, 1943), apparently had no such damaging effect on the kidneys. Fisher et al. (1961) observed repeated outbreaks in both male and female yearlings when transferring them from floor pens to individual cages. These authors considered water deprivation and change in environment as a possible sole or major cause.

Investigating the popular claim that avian monocytosis represented a form of wheat poisoning, Quigley (1943) subjected it to experimental inquiry and obtained some epizootiologic as well as experimental support for this belief (1944a). He failed to find chemical differences between pullet disease-inducing and non-inducing wheat samples, but found the former to have lowered germination ability (1944b) and to be associated with a high bacterial and low fungal flora (Petty and Quigley, 1947). In further

studies Quigley (1948) again found different loss of wheat to vary in their ability to cause pullet disease. Fortification of the mash with calcium carbonate and sodium bicarbonate improved egg production but had no preventive effect on the disease.

This subject of possible causes of visceral gout in birds has been reviewed by Stonebrink (1947).

High atmospheric temperatures have long been considered as a possible factor, as indicated by the synonym "summer disease." Yeates et al. (1941), in their studies of the reactions of domestic fowl to hot atmospheres, failed to observe pullet disease. Jungherr, in cooperation with Scott and Matterson (1946), examined chickens which had been kept at constant high atmospheric temperatures with or without adequate water supply, but failed to find a condition which would fit the diagnostic criteria of avian monocytosis. Fox (1951) reviewed the problem of heat tolerance of domestic fowl and found the survival time to depend on breed differences and persistency in water consumption. Van Ness (1953) collected nationwide incidence data which suggested bluecomb in chickens to resemble early shock and to occur primarily 3 to 4 days following temperature waves of 85° F. or over.

Maas and Voûte (1961) made detailed epizootiologic observations in an intensive breeding area of southwestern Holland during the summer of 1959. In a pullet population at risk of 800,000, 4 to 8 months old, with flocks averaging 5,000 birds on 10 acres of land, they observed 215 cases of bluecomb, 124 of them uncompli cated. The attack rate was 3 to 4 per cent of young hens but was not uniform since broad strips were apparently spared. Clinical signs corresponded to those in the world literature. Outbreaks were frequently preceded by sudden changes in temperature. Egg production receded by 30 per cent and returned to normal within 3 to 6 weeks after treatment. The latter consisted of reduction of grain feeding and 1:10,000 oxytetracycline in the drinking water. This recent report is of interest in view of the diminishing incidence in the U.S.A.

No bacterial organism has been found to be constantly associated with avian monocytosis, except for the unconfirmed claim of Weisner (1941) that the disease is caused by certain strains of Escherichia coli.

Waller (1942, 1944a) reported the isolation of a filterable agent from the blood, liver, feces, and eggs of birds affected with the acute form. The virus (1944b, 1945) could be cultivated on the chorio-allantoic membrane of 8- to 9-day-old chicken embryos where it produced a compact or circular lesion with radiating processes, stunting of the embryo, and death in about 12 per cent. Turkey and duck embryos usually succumbed to the inoculation. Injection or feeding of the freshly isolated virus caused a nonfatal sickness (in about 50 per cent) characterized by subcutaneous edema, widespread petechiation, tumefaction of the parenchymatous organs, and catarrhal enteritis. The experimental disease in chickens and turkeys was accompanied by marked heterophile leukocytosis which reached its peak about 96 hours post inoculation.

Blood sera from birds that had recovered from the experimental or spontaneous diseases were capable of agglutinating washed killed Salmonella pullorum organisms which had been allowed to adsorb virus from infected allantoic fluid.

Based on the refractivity of birds to reinoculation, a live vaccine was prepared from infected chorio-allantoic membranes dried in vacuo over anhydrous calcium chloride and used on about 44,000 birds with encouraging results.

Isolation of a virus in embryonated chicken eggs was reported by Watanabe and associates (1952a, b). The authors succeeded in the isolation of two strains from Chamberland L_3-filtered intestinal content of acutely affected birds. The virus was cultivated in the allantoic cavity of 8- to 10-day-old chicken embryos for 14 passages and caused embryonic deaths within 1½ to 3 days, accompanied by hyperemia and petechiation of eyelids,

legs, and lung, or severe stunting within 7 days. Later studies found the yolk sac route preferable. The egg-propagated virus was nonhemagglutinating and neutralizable, and was capable of inducing the field syndrome in healthy birds on oral or intravenous administration within 5 to 6 days. Tanaka and Kawashina (1961) further characterized the virus of infectious diarrhea of pullets. Blaxland (1957) confirmed the embryo-pathogenicity of the Japanese agent when inoculated into the yolk sac of 6-day-old embryos. But intravenous inoculation of the virus into British fowl caused only a transient monocytosis. Neutralization tests on the principal birds prior to, and post, inoculation were not reported.

Although the viral etiology has not been confirmed definitely, failure to do so may be entirely technical in nature but demonstrates the difficulties involved, particularly for diagnostic purposes. Waller (1944b) rightly pointed out the unlikeliness of many birds being attacked simultaneously by uremia of noninfectious origin. On the other hand, to explain the often explosive outbreaks of avian monocytosis on an infectious basis, one would have to assume that the viral agent is already widely seeded in the susceptible population, and that its pathogenic action is set into motion by secondary nonspecific factors (Jungherr and Matterson, 1941).

Treatment and control. Certain therapeutic measures have been used in the field, apparently with favorable response if applied in the early stages of the acute form of the disease. For individual treatment Van Ness (1951a, b) suggested instillation into the crop of 10 to 20 ml. of 4 per cent acetic acid or of vinegar, followed by gentle massage.

In general, the measures consist in providing an abundance of clean, readily available water, and cool, well-ventilated quarters, and in reducing consumption of grain. If possible, birds should be allowed access to runways or shaded range. Flushes are contraindicated in view of the severe dehydration.

Some observers claim to have obtained good results from the use of 1:2,000 copper sulfate or 1:1,000 potassium dichromate in the drinking water (Weisner, 1941). Molasses has been used widely and has some justification because in high doses it prevents experimental nephrotoxicoses probably on account of its potassium content (Scott et al., 1944). Blaxland (1957) thinks of molasses merely as a source of readily assimilated carbohydrate. Molasses may be used either in the drinking water (2 per cent) or administered in a mash composed of equal parts of bran and rolled oats, mixed with 10 to 30 per cent of molasses and water in amounts sufficient to obtain a crumbly consistency. The treated mash is given on alternate days for a period of 3 hours, after withholding food for about 2 hours.

Instead of molasses, potassium chloride or good fertilizer grade of muriate of potash (containing at least 60 per cent K_2O) may be used at the rate of 0.5 per cent in the water for the first 7 days and, if necessary, at the rate of 1¼ per cent in the feed for an additional 7 days. According to Van Ness (1917a, b), under experimental conditions, prolonged or excessive use of potassium chloride may have untoward effects. The general role of potassium in human medicine is finding new emphasis (Darrow, 1950; Martin et al., 1951) and militates against a concept of pullet disease as being a primary potassium deficiency.

Antibiotics, namely penicillin, chlortetracycline, and oxytetracycline, were first shown to be effective in individual oral treatments of turkey cases by Peterson and Hymas (1951), who also obtained good results in the field with 100 and 5 parts per million of oxytetracycline in mash and water, respectively. Since then, treatment of the disease in chickens with high level oxytetracycline has been reported as both successful (Cromley, 1953) and unsuccessful (Adler et al., 1952). Pullet disease responded promptly to treatment with high levels of chlortetracycline in the experience of McKay and

Pelly (1956). On the theory that the acute form is a metabolic disorder and the sub-acute form is due to an infectious agent, Blaxland (1957) recommended treatment only for the subacute form. He stated, however, that this was based upon field experience, not upon controlled experiments.

If the virus etiology of avian monocytosis is confirmed, specific vaccination may be contemplated. At the present time, a specific vaccine is not available.

Control measures should be attempted along the lines of good management. Direct and indirect contact with affected birds should be avoided. Routine prophylactic vaccinations (fowl pox, etc.) are best carried out during the early growing period, preferably at the age of 2 months. At the time of housing, the birds should be well fleshed but not fat; transfer from the range to confinement must be made gradually with a minimum of disturbance to the birds. Shade, ventilation, and water supply are important factors, and mash feeding should be intermittent during the critical period.

INFECTIOUS NEPHROSIS OF BROILERS

An apparently new disease of chickens was observed in the Delmarva broiler area since 1956 and reported by Cosgrove (1962) under the term avian nephrosis, popularly known as "Gumboro disease" according to the locale. Referring to it as "nephritis-nephrosis syndrome of chickens" Winterfield and Hitchner (1962) determined its viral etiology but found two agents which produced mild respiratory signs associated with renal alterations. Winterfield et al. (1962) recognized distinct pathogenetic differences between these agents and suggested naming the respiratory agent "infectious bronchitis variant virus" and the Gumboro disease agent which causes enlargement of the bursa of Fabricius and renal lesions, "infectious bursal agent."

The immunologic relationship of the bronchitis variant virus to other known serotypes of this virus has been detailed by Hitchner et al. (1964) and Winterfield et al. (1964).

The pathologic aspects of the infectious bursal agent have been studied by Helmboldt and Garner (1964). After intraocular inoculation of 21-day-old susceptible chicks, there was gross enlargement of the bursa of Fabricius within 3 days and microscopically general necrosis of the lymphoid elements, especially in the spleen, cecal tonsils, and thymus followed by partial regeneration. Nephritis occurred irregularly in the terminal phases of the experimental disease.

In Australia, Newton and Simmons (1963) likewise observed an acute disease of broilers with initial respiratory and late nephritic signs and believed it to be the most prevalent form of avian nephritis in Queensland. The authors obtained a chicken embryo lethal agent via the yolk sac route. Suspensions of kidneys from affected birds or of infected yolk sac reproduced the disease either by intra-abdominal inoculation or exposure to aerosols. By the latter techniques two-day-old chicks developed a nephritic, older chicks a respiratory, syndrome. In the over-all, the disease was similar to that described by Hungerford (1962) as fulminating "uraemia" in brooder plants.

Cumming (1963a) objected to the term uremia and stated that the "kidney breakdown disease complex" has been a problem in Australian poultry flocks since 1948 and now represents a major poultry disease. In six-week-old chicks the disease started with respiratory signs for 24 hours, followed in 3 to 4 days by high losses (up to 15 per cent) which were principally characterized by dehydration and renal enlargement. Inoculation of pathologic material into the allantoic cavity of developing hen's eggs yielded a virus which caused changes in the embryos ordinarily ascribed to those of infectious bronchitis virus, and which was capable of reproducing the disease in 15-day-old chickens. Since Australia was believed to be free from avian viral bronchitis the isolant was con-

sidered an aberrant strain of infectious bronchitis virus.

In follow-up studies on infectious avian nephrosis in Australia, Cumming (1963b) reported isolation of the virus from various localities around Sydney and elsewhere. The virus could be isolated from lung, trachea, and kidney, even from dead birds. The experimental disease, produced by intraocular instillation of infected allantoic fluid, caused respiratory signs without discharge in 24 hours. After a 3- to 4-day period of normalcy, the birds became hunched, ate little, and drank much. The vents were soiled, the droppings white, and the combs cyanotic. The natural disease was also observed in young layers where respiratory signs were followed within 4 days by drop in egg production, embryonic mortality in their progeny for about 2 weeks, and decrease in egg quality for a longer period, by and large typical of infectious bronchitis. An attack was followed by immunity in about 6 weeks. The disease was considered self-limiting and probably caused by interaction of nutritional and viral factors. Except for the enlargement of the bursa of Fabricius, the Australian nephrosis syndrome resembled the American form; preliminary results have shown no serologic cross-neutralization of the respective viruses.

While the interrelationships of avian nephrosis in broilers in various parts of the world to pullet disease in older birds have not been worked out, the recent findings strengthen the infectious theory and promise etiologic clarification. They also demand careful re-examination of bronchitis-like viral strains, especially vaccine strains, for purity and pathogenetic spectra.

BLUECOMB DISEASE OF TURKEYS

Occurrence. Bluecomb disease of turkeys has been recognized in various turkey raising areas of the United States and Canada. Peterson and Hymas (1951) described an unfamiliar disease of turkeys, observed in the state of Washington for the past seven years and locally known as "Mud Fever," which had the general characteristics of pullet disease. Pomeroy and Sieburth (1953) reported on an extensive outbreak of the disease that occurred in Minnesota in 1951. A similar condition had been recognized as occurring sporadically in growing turkeys for several years. It had been designated as trichomoniasis of the lower intestinal tract because of the presence of increased numbers of trichomonads in the cecum and rectum. Boyer (1953) reported on the disease in New York as having a similar pattern to what was described in Minnesota. The disease was recognized in Virginia as a serious disease problem in young turkeys (Sieburth and Johnson, 1957). Ferguson (1961) reported the disease had occurred in Ontario for several years and in 1959 had become a serious disease problem.

Economic importance. Surveys of turkey growers conducted by the State-Federal Crop and Livestock Reporting Service in Minnesota in 1951, 1956, and 1961 indicated that bluecomb disease was the most costly disease encountered by the turkey industry in that state. In 1961, 31 per cent of the flock owners reported losses from the disease, in 1956, 22 per cent, and in 1951, 14 per cent. The estimated value of the turkeys lost in 1961 was $189,000 and morbidity losses would greatly increase that figure. The average age of the birds at the time of most frequent loss in 1951 was 21 weeks, in 1956 it was 13 weeks, and in 1961 it was 9 weeks. Because of the change in turkey production to a year-round program on many farms today, the disease is encountered frequently in young birds before they are placed on range, as compared to previous years when the production was more seasonal and the disease was seen in mature birds (Erlandson and Mesick, 1962).

Etiology. The exact cause of bluecomb disease in turkeys has not been fully characterized. Pomeroy and Sieburth (1953) demonstrated the infectious nature of the disease and were able to reproduce the disease with unfiltered intestinal contents. Tissues, other than the intestinal

tract, did not harbor the transmissible agent. Sieburth (1954) studied various bacterial isolates from the intestinal tract of bluecomb disease-infected poults but was unable to reproduce the disease with various types of microorganisms. The agent failed to pass Seitz EK, Berkefeld N, and sintered glass UF filters. Sieburth and Pomeroy (1955) reported additional studies on the etiology but were unsuccessful in isolating a specific agent. Tumlin et al. (1957) established that the agent readily passed Berkefeld and Selas filters of the smallest pore size but would not pass through a Seitz EK filter. Sieburth and Johnson (1957) reported similar findings. A similar agent has been obtained from bluecomb-affected chickens which is transmissible to chicks and poults. However, infective material from poults did not cause the disease in chicks (Sieburth and Pomeroy, 1956b). Tumlin and Pomeroy (1958) found that 1:2 dilutions of serum from recovered field cases completely neutralized Selas 02 filtrates of agent-bearing intestinal contents, but no evidence of measurable passive immunity was found in their progeny. Truscott et al. (1960) isolated a small Gram-negative anaerobic pleomorphic rod from the intestinal tract, which they considered as the etiological agent. Truscott and Morin (1964) further characterized the agent as a member of the genus Vibrio, and reproduced the disease with vibrio cultures. Vibrio isolations were made from the liver and bile, as well as intestinal tract. Serological studies suggested that at least three serological types were isolated. The relationship of these vibrios to the vibrio associated with hepatitis in chickens has not been established.

Larsen (1964) has consistently isolated an enterovirus from intestinal tracts of bluecomb-infected turkeys and recently has verified the presence of vibrio organisms in experimental and natural infections. He has not been able to reproduce the disease with the enterovirus or vibrio organisms alone or in combination.

Symptomatology. The disease affects turkeys of all ages. In young poults the disease appears suddenly. There is depression, subnormal body temperature, anorexia, dehydration, and frothy or watery diarrhea. There is constant chirping and poults seek heat. The incubation period is 48 to 72 hours. In growing turkeys the appearance of the disease in a flock is sudden with a concurrent drop in water and feed consumption. The affected turkeys have a diarrhea, some depression and show darkening of the head and skin. There is a rapid loss of weight and a sunken crop. The droppings may contain mucous threads and casts and may be greenish to brownish in color. As the disease progresses in a flock, the droppings contain primarily urates. There is a subnormal body temperature. In turkeys in production a similar pattern as seen in growing turkeys is encountered and, in addition, a rapid drop in egg production with chalky eggshells.

The mortality varies depending on the age of the turkeys and environmental factors. In young poults under experimental conditions the loss may vary from 50 to 100 per cent, whereas under field conditions, the losses may vary from 5 to 50 per cent with higher losses occasionally encountered. In young turkeys four to eight weeks of age the death loss may be kept to a minimum if supplemental heat is provided. In range turkeys the loss may be low but is dependent on environmental conditions. If adverse weather conditions prevail, the loss may be as high as 25 per cent. The course of the disease may extend over a period of ten days to two weeks and it may require several weeks for the birds to regain lost weight. In mature birds, particularly males, some never regain satisfactory weight and there is a general unevenness in the flock. The morbidity loss may be high because of the loss of weight and inability of the flock to mature at a normal rate (Pomeroy and Sieburth, 1953; Sieburth, 1954; Sieburth and Pomeroy, 1955).

Pathology. In poults the gross changes

are confined to the intestinal tract. Contents of the duodenum and free portion of the small intestine and cecums are watery and gaseous. Bulbous areas are noted along the intestinal tract. There are no gross lesions in the lungs, heart, liver, spleen, pancreas, and kidneys. The muscles are dehydrated with a generalized emaciated condition of the carcass.

In growing and mature turkeys the gross changes are confined primarily to the intestinal tract, although changes are noted in some of the other organs. The liver is normal in size and appearance. The spleen is usually smaller than normal. The pancreas may appear normal or have a chalky appearance with numerous small whitish areas. The kidneys usually present a normal appearance. Urate deposits are rarely found. The crop is empty. The lumen of the intestinal tract contains watery, gelatinous mucus and occasionally casts. The cecums are distended and filled with watery yellowish-brown feces having a fetid odor. Small petechial hemorrhages may be noted on the surface of the intestinal mucosa (Pomeroy and Sieburth, 1953; Sieburth, 1954; Sieburth and Pomeroy, 1955). Hilton (1954) found similar histopathological changes in tissues from experimentally and spontaneously infected turkeys. Histologically changes were primarily of a catarrhal enteritis. He found a marked round cell infiltration of the adrenals.

Hematology. Hilton's (1954) hematologic findings in experimentally infected turkeys were similar to those reported in chickens (Jungherr and Matterson, 1944).

Chemical pathology. Hilton (1954) conducted some blood chemistry studies on experimentally infected turkeys but the results were inconclusive.

Differential diagnosis. In young poults the disease may be easily confused with hexamitiasis. Salmonellosis may be a complicating factor in natural outbreaks. Moniliasis is a very common secondary infection particularly following antibiotic therapy. In growing and mature birds increased numbers of trichomonads are found in the contents of cecums and rectums. Their role in natural outbreaks is not known. The possibility of known specific infections, such as erysipelas, fowl cholera, and entero-hepatitis, must be eliminated by laboratory studies.

Treatment and control. Because of the highly infectious nature of the disease every management precaution must be taken to prevent the introduction of the disease onto a turkey farm. Since there is evidence of a carrier status in recovered turkeys, complete depopulation of a turkey farm is the only way to break the cycle of infection.

Various antibiotics and other chemotherapeutic agents have been evaluated in the treatment of the disease. No regime has been found that will completely prevent the disease. Peterson and Hymas (1951) reported success in individual oral treatment of turkeys with penicillin, chlortetracycline, and oxytetracycline. Various antibiotics have been used under controlled conditions at high levels. Penicillin, chlortetracycline, oxytetracycline, and streptomycin were effective in reducing the death loss at 500 grams per ton in the feed and 1.0 gram per gallon of drinking water (Pomeroy and Sieburth, 1953; Sieburth and Pomeroy, 1956; Pomeroy, 1956a, b). Tumlin and Pomeroy (1958) reported on the prophylactic value of three nitrofurans in experimental infections that resulted in reduced mortality but had no effect on morbidity.

Truscott *et al.* (1960) found the bacterium they considered the causative agent of bluecomb disease resistant to penicillin, streptomycin, tetracycline, erythromycin, chloromycetin, and spotin, but sensitive to neomycin. They recommended 0.7 gm. of neomycin per gallon of drinking water as a treatment.

In turkeys sick with this disease, increasing the environmental temperature until the birds seem comfortable is also of considerable value in keeping losses to a minimum.

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Chapter 31: BLUECOMB DISEASE OF TURKEYS 861

————, and Levine, J. M.: 1940. The pathologic concept of so-called "pullet disease." Poultry Sci. 19:554.

————, and Levine, J. M.: 1941. The pathology of so-called pullet disease. Am. Jour. Vet. Res 2:261.

————, and Matterson, L. D.: 1944. Avian monocytosis, so-called pullet disease. Proc. 48th Ann. Meet. U.S. Livestock Sanit. Assn., p. 185.

————, Scott, H. M., and Matterson, L. D.: 1946. Unpublished data

Larsen, C. T.: 1964. Unpublished data. University of Minnesota.

Levine, J. M., and Jungherr, E.: 1941. Unpublished data.

Lindtner, M.: 1960. Avian monocytosis. Vet. Inspector (Australia) 61.

Lucas, A. M.: 1947. Intranuclear inclusions in the islands of Langerhans of chickens. Am. Jour. Path. 23:1005.

————: 1951. Occurrence of two types of intranuclear inclusions in the pancreas of turkeys, of which one suggests a virus infection. Poultry Sci. 30:655.

Maas, H. J. L.: 1960. Een beschouwing over de blauwe kam ziekte bij hoenders. Vlaams Diergeneesk. Tijdschr. 29:255.

————: 1961. De invloed van blauwe kam ziekte op het eigewicht en de eikwaliteit, een onderzoek met behulp van praktijkmethoden. Vlaams Diergeneesk. Tijdschr. 30:269.

————, and Voûte, E. J.: 1961. Een praktijkonderzoek over de blauwe kam ziekte bij hoenders in "het land von Weert." Vlaams Diergeneesk. Tijdschr. 30:106.

McKay, W. M., and Pelly, A.V.: 1956 The treatment of "pullet disease" in fowls. Brit. Vet. Jour. 112:70.

Martin, H. E., Reynolds, T. B., Snyder, E. N., Berne, C. J., Homann, R. E., Jr., Edmondson, H., Blatherwick, N., Fields, I., Wertman, M., and Westover, L.: 1951. Etiology and treatment of serum potassium deficits. Jour. Am. Med. Assn. 147:24.

Mochiruki, H.: 1951. Private communication. (For further references see also Vet. Bul. 1954, 24:437.)

————, Surawa, Y., Shibata, D., and Miyaiti, K.: 1952. Pathological studies on a diarrhea of fowl (avian monocytosis). 24th Exper. Rep. Govt. Exper. Sta. for Anim. Hyg., pp. 39–48.

Moultrie, F., Cottier, G. J., and King, D. F.: 1955. Additional evidence for genetic variation in resistance to "blue comb" disease. Poultry Sci. 34:458.

Newton, L. G., and Simmons, G. C.: 1963. Avian nephritis and uraemia. Austral. Vet. Jour. 39:135.

Olson, C., Jr.: 1959. Avian hematology. In H. E. Biester and L. H. Schwarte, Diseases of Poultry. 4th Ed. Iowa State University Press, Ames, Iowa, Pp. 53–69.

Oppenheimer, E. H.: 1941. The lowering of blood uric acid by uricase injections. Bul. Johns Hopkins Hosp. 58:190.

————, and Kunkel, H. G.: 1943. Further observations on the lowering of blood uric acid by uricase injections. Bul. Johns Hopkins Hosp. 73:40.

Peterson, E. H., and Hymas, T. A.: 1951. Antibiotics in the treatment of an unfamiliar turkey disease. Poultry Sci. 30:466.

Petry, A. M., and Quigley, G. D.: 1917. The microflora of wheat feeds as related to the incidence of blue comb in chickens. Poultry Sci. 26:7.

Pomeroy, B. S.: 1956a. High level use of antibiotics. Proc. 1st Internat Conf. on the Use of Antibiotics in Agr., Publ. 397, Nat. Acad. Sci., Nat. Res. Council, p. 55

————: 1956b. Use of furazolidone and antibiotics in bluecomb disease of turkeys. Proc. 1st Nat. Symposium on Nitrofurans in Agr., Michigan State Univ., East Lansing, p 75.

————, and Sieburth, J. M.: 1955. Bluecomb of turkeys. Proc. Book Am. Vet. Med. Assn., p 321.

Quigley, G. D.: 1943. Is blue comb of fowls produced by wheat? Poultry Sci. 22.267.

————: 1944a. The effect of wheat upon the incidence of pullet disease or blue comb. Poultry Sci. 23.386.

————: 1944b. Germination differences of wheat natured in a study of pullet disease. Poultry Sci. 23.547.

————: 1948. Further studies with wheat and pullet disease. Poultry Sci. 27.617.

Quereli, S. H.: 1955. The occurrence in West Pakistan of a disease resembling avian monocytosis or pullet disease in America. Jour. Am. Vet. Med. Assn. 127:451.

Ranby, P. D.: 1958. Bluecomb strikes pullets in lay. Queensland Agr. Jour. 84.435.

Ryff, J. F., and Staseth, H. J.: 1942. A cholera-like disease of poultry. Vet. Med. 37:291.

Scott, H. M., Jungherr, E., and Matterson, L. D.: 1944. Possible role of potassium in pullet disease. Proc. Soc. Exper. Biol. and Med. 57.7.

————, Matterson, L. D., and Jungherr, E.: 1946. Unpublished data.

Selye, H.: 1942. Production of nephrosclerous by overdosage with desoxycorticosterone acetate. Canad. Med. Ann. Jour. 47:515.

————: 1943. Production of nephrosclerous in the fowl by sodium chloride. Jour. Am. Vet. Med. Assn. 103.140.

————, and Stone, H.: 1943. Role of sodium chloride in production of nephrosclerous by steroids. Proc. Soc. Exper. Biol. and Med. 52.190.

Sieburth, J. McN.: 1954. Bluecomb disease of turkeys: Antibiotic prophylaxis and etiology. Ph.D. Thesis. Univ. Minnesota.

Sieburth, J. McN., and Johnson, E. P.: 1957. Transmissible enteritis of turkeys (blue comb). Poultry Sci. 36:256.

——, and Pomeroy, B. S.: 1956a. Bluecomb disease of turkeys. II. Antibiotic treatment of poults. Jour. Am. Vet. Med. Assn. 128:509.

——, and Pomeroy, B. S.: 1956b. Experimental transmission of a catarrhal enteritis of chicks similar to bluecomb disease. Am. Jour. Vet. Res. 17:24.

——, and Pomeroy, B. S.: 1958. Bluecomb disease of turkeys. III. Preliminary studies on etiology. Proc. Book Am. Vet. Med. Assn., p. 301.

Soller, W. G.: 1959. Avian nephritis and visceral gout. Lab. Investigation 8:1319.

Spanner, R.: 1925. Der Pfortaderkreislauf in der Vogelniere. Gegenbauers morphologisches Jahrb. 54:560.

Stonebrink, B.: 1947. De pathogenese van jicht bij vogels. Tijdschrift voor Diergeneesk. 72:164.

Tanaka, F., and Kauashina, H.: 1961. Some properties of the virus isolated from pullets with so-called infectious diarrhoea-like disease (translated title). Japanese Jour. Vet. Sci. 23:468.

Thompson, J. J.: 1951. Antihistamine therapy in pullet disease (avian monocytosis). Austral. Vet. Jour. 27:291.

Truscott, R. B., Connell, M. C., Ferguson, A. E., and Wills, C. G.: 1960. A bacterial agent causing bluecomb disease in turkeys I. Isolation and preliminary laboratory investigations, Avian Dis. 4:391.

——, and Morin, E. W.: 1964. A bacterial agent causing bluecomb disease in turkeys. II. Transmission and studies of the etiological agent. Avian Dis. 8:27.

Tumlin, J. T., and Pomeroy, B. S.: 1958. The prophylactic effect of Nitrofurans in feed on bluecomb disease mortality and weight gains in day-old poults. Proceedings of 2nd National Symposium on Nitrofurans in Agriculture. P. 144.

——, and Pomeroy, B. S.: 1958. Bluecomb disease of turkeys, V. Preliminary studies on parental immunity and serum neutralization Am. Jour. Vet. Res. 19:725.

——, Pomeroy, B. S., and Lindorfer, R. K.: 1957. Bluecomb disease of turkeys. IV. Demonstration of a filterable agent. Jour. Am. Vet. Med. Assn. 130:560.

Vaubhnar, T. N., and Parulkar, D. T.: 1961. Note on pullet disease in Bombay. Bombay Vet. College Magazine 9:55.

Van Ness, G.: 1947a. Potassium as an excitant to blue comb. Poultry Sci. 26:557.

—— 1947b. The production of so-called pullet disease. Poultry Sci. 26:304.

—— 1951a. Symposium on pullet disease. Proc. Ann. Meet. Poultry Sci. Assn., Knoxville, Tenn.

—— 1951b. Stasis of crop in blue comb. Jour. Am. Vet. Med. Assn. 118:106.

—— 1953. Weather influence in blue comb in chickens. Science 118,601.

Wachstein, M., and Meisel, E.: 1951. Nephrotoxic action of dl-ethionine. Proc. Soc. Exper. Biol. and Med. 77:648.

Waller, E. F.: 1942. Isolation of a filtrable virus from chickens affected with blue comb disease. Science 95:560.

—— 1942a. Virus etiology of blue comb disease. Proc. Sixteenth Ann. Conf. Lab. Work. in Pullorum Disease Control, Univ. Conn. Coll. Agr., Mimeo. Rep.

—— 1944b. Blue comb disease Proc. 48th Ann. Meet. U.S. Livestock Sanitary Assn., p. 171.

—— 1945. Blue comb disease. N.H. Agr. Exper. Sta., Tech. Bul. 85:3.

——, Tepper, A. E., Halpin, R. B., and Davis, H. A.: 1942. The etiology, pathology, and prevention of contagious indigestion. N.H. Agr. Exper. Sta. Rep., Bul. 345:50.

Watanabe, M.: 1952a. Avian infectious diarrhoea. I. Clinical, hematologic and histopathologic findings. II. Transmissible experiments and isolation of the causative agent. Jap. Jour. Vet. Sci. 14:263.

——, Yamano, T., Mifune, R., and Oochi, T.: 1951. An avian diarrhea (strictly similar to avian monocytosis). I Clinical findings and blood picture. II. Isolation of the causal agent (a virus). (In Japanese with English translation) Jap. 32nd Meet. Jap. Vet. Med. Assn., Sept. 11.

——, Yamano, T., Mifune, R., and Oochi, T.: 1952b. Avian infectious diarrhea (similar to so-called pullet disease). (In Japanese with English summary.) Virus 2:99.

Weaver, G. H.: 1941. Bright's disease in the avian subject. Proc. 14th Ann. Conf. Lab. Work. in Pullorum Disease Control, Federal Dept. Agr., Mimeo. Rep., Ottawa, Canada.

Weiner, E. S.: 1911. How the veterinarian can develop a poultry practice. Mich. St. Coll. Vet. 1:10.

Wilenuky, A. O.: 1939. Occurrence, distribution, and pathogenesis of so-called liver death and/or the hepatorenal syndrome. Arch. Surgery 38 625.

Winterfield, R. W., and Hitchner, S. B.: 1962 Etiology of an infectious nephritis-nephrosis syndrome of chickens. Am. Jour. Vet. Res. 23 1273

——, Hitchner, S. B., Appleton, G. S., and Cosgrove, A. S.: 1962. Avian nephrosis, nephritis and Gumboro disease. L & M News and Views, L & M Laboratories, Selbyville, Delaware 3(5).

——, Hitchner, S. B., and Appleton, G. S.: 1964 Immunological characteristics of a variant of infectious bronchitis virus isolated from chickens. Avian Dis. 8:40.

Yeates, N. T. M., Lee, D. H. K., and Hines, H. J. G.: 1941. Reactions of domestic fowls to hot atmospheres. Proc. Roy. Soc. Queensland 53:105

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32

Neoplastic Diseases of the Chicken

An attempt is made in the following presentation to describe briefly the various forms of neoplastic disease that occur in the chicken and to discuss their salient characteristics. This contribution is intended to supply the information necessary for a pathologist to make a differential diagnosis of neoplasia in the chicken, provided the case in question is one of the commoner forms of tumor. Key references to the literature are provided for those who wish to seek more detailed information.

Scientific interest was aroused about 55 years ago in two types of neoplasia of the chicken which were found to be transmissible by means of an ultramicroscopic agent separable from tumor cells. These tumors were fowl leukosis and certain tumors of connective tissue. Because of considerable research on the transmissible tumors of chickens, evidence is accumulating to indicate contact exposure of the very young bird is the mode of transmission of these diseases under natural conditions.

INCIDENCE

Although neoplastic disease is generally recognized as one of the more common diseases of the domestic chicken, its incidence can be estimated in only a general manner. Reports referred to in Table 32.1 indicate an incidence of from 3 to 19 per cent.

Ask-Upmark (1938) reported what he believed to be an epidemic of tumors in a flock of chickens in Sweden. Between 20 and 25 birds from a flock of 100 died within a year. Only 5 of the birds were actually examined, yet all 5 were affected with carcinoma of the ovary with extension to the viscera. Olson (1912) studied the tumors that occurred in a small poultry flock in which the incidence of disease

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TABLE 32.1

Frequency of Neoplasia Observed in Chickens
(Excluding Neurolymphomatosis and Osteopetrosis)

Author	Locality	Number of Birds Examined	Period of Observation	Per Cent With Tumor
Curtis (1915)	Maine	880	8 years	8.98
Schneider (1926)	England	11,000	. . . .	2 to 3
Hoogland (1929) . . .	Holland	1,707	32 years	10
Babic (1931) .	Yugoslavia	647	8 years	6.6
Eber and Malke (1932)	Germany	11,903	32 years	3.12
Goss (1940), .	New York	7,408	. . . . .	19.5
Olson and Bullis (1942) . . .	Massachusetts	2,304	2 years	12 9
Campbell (1945) .	Scotland	2,063	5 years	18.7
Reis and Nobrega (1955). .	Brazil	15,549	23 years	6.6

was unusually high. The flock numbered 48 birds, and when the last bird was slaughtered, 3 years later, 13 birds (27 per cent) had died with neoplastic disease. In another flock of 478 birds, 42, or 11.4 per cent, became affected with lymphocytoma in a 2-month period (Olson, 1948).

Various factors obviously will affect the result of any survey on the incidence of neoplasia. Some of the more important factors are age of the group under survey, length of period covered by the survey, the *genetic composition or inheritable tendencies* of the group under survey, and certain other factors, as yet unknown, which may cause a high incidence of certain *types of tumors.*

Some forms of neoplasia are much more common than others. Lymphocytoma is recognized generally as the most common tumor found in chickens. Collectively the other varieties cause much loss, and other types of tumors may be a serious problem in some flocks of poultry.

CLASSIFICATION OF NEOPLASMS OF CHICKENS

Many schemes or systems have been proposed for the systematic grouping of true tumors. *A fairly satisfactory system of classifying tumors is that which depends on the type cell from which the tumor originates* (Table 32.2). Such a classification is based on the embryogenesis and histologic identity of the respective cells that make up normal tissues and takes cognizance of the fact that tumors may arise from any of the distinct varieties of cells that are concerned normally in the structural or functional welfare of the body. One practical difficulty with this system of classification is that although cells of normal or mature tissues usually possess characteristics by which they can be recognized readily, unripe or immature cells such as make up rapidly growing tumors, or those cells representing primitive tissues, may exhibit so little differentiation that recognition of the type cell may be difficult or impossible.

TUMORS OF CONNECTIVE TISSUE

The widespread distribution of the different connective tissues throughout the body provides potential sources for a variety of connective tissue tumors. Another potential source of connective tissue tumors *is the free histiocyte which exists in* all loose connective tissues.

Tumors derived from the various con-

TABLE 32.2
CLASSIFICATION OF NEOPLASMS OF CHICKENS

I. Tumors of connective tissues
 	
A. Benign
    Fibroma
    Myxoma
    Lipoma
    Osteoma
    Chondroma
 	
B. Malignant
    Fibrosarcoma
    Myxosarcoma
    Liposarcoma
    Osteogenic sarcoma (osteochon-
      drosarcoma)
    Chondrosarcoma
    Histiocytic sarcoma
 	
C. Special forms of connective
   tissue tumors
    Neurogenic sarcoma
 	
II. Tumors of muscle tissue
    Leiomyoma
    Rhabdomyoma
 	
III. Tumors of blood and lymph channels
    Hemangioma
    Lymphangioma
 	
IV. Tumors of hemoblastic origin
    Lymphocytoma
    Myelocytoma
    Leukosis
	V. Pigmented tumors
	    Melanoma
	
	VI. Tumors of nerve tissue
	    Glioma
	    Neuroblastoma
	    Retinoblastoma
	    Ganglioneuroma
	
	VII. Tumors of epithelial tissues
	
	A. Benign
	    Papilloma
	    Adenoma
	
	B. Malignant
	    Carcinoma
	
	C. Special forms of epithelial tumors
	    Hypernephroma
	    Arrhenoblastoma
	    Dysgerminoma
	    Granulosa-cell tumor
	
	VIII. Tumors of serous membranes
	    Mesothelioma
	
	IX. Mixed tumors
	    Thymoma
	    Carcinosarcoma
	    Teratoma
	    Dermoid cysts
	    Embryonal nephroma

nective tissues may be extremely simple in their constituents and easily recognized, or they may be more or less complex in structure and difficult to classify with certainty. Many variations of structure occur. Single benign fibroblastic tumors may be associated with edema or mucinous substances, and it may be difficult in the latter instance to determine whether or not one is dealing with a simple fibroblastic entity associated with mucinous degeneration or with a tumor that is primarily myxomatous. The connective tissue tumors composed of cartilage, fat, or bone are ordinarily not difficult to recognize if sufficient differentiation has occurred.

Frequency of occurrence. Generally speaking, the benign connective tissue tumors are among the rarer neoplasms of chickens. In a series of 113 chicken tumors (exclusive of leukotic tumors) reported by Goss (1940a), only 1 benign connec-

tive tissue tumor – a fibroma – was listed. Among 237 tumors of chickens examined histologically by Eber and Malke (1932), 11 benign connective tissue tumors were found. These included 1 myxoma, 5 fibromas, and 5 lipomas. Hoogland (1929), of Utrecht, reported 12 fibromas among 176 chicken neoplasms. No other forms of benign connective tissue tumors were listed by Hoogland. Eight of Goss's series of 113 tumors were fibrosarcomas. In Jackson's (1936a) series of 205 neoplasms (including lymphocytoma and leukosis) of chickens, 21 malignant connective tissue tumors are listed. The different varieties were as follows: 7 fibrosarcomas, 1 myxosarcoma, 1 osteochondrosarcoma, and 15 histiocytic sarcomas. In the series of 585 neoplasms of chickens, inclusive of leukosis, presented by Olson and Bullis (1942), there were 16 fibrosarcomas, 5 histiocytic sarcomas, 5 neurogenic sar-

comas, 1 osteochondrosarcoma, and 1 fibrochondrosarcoma.

As in most other forms of neoplasia of the chicken, adequate, precise statistical data on the incidence of connective tissue tumors are not available. In the older literature the term "sarcoma" was used in a rather nonspecific sense, and no attempt was made to separate the various types of sarcomas on the basis of the type cell. Our observations and the impressions obtained from the reports of others suggest that the benign connective tissue tumors occur infrequently and that the malignant varieties are at least of moderate frequency.

Sites of occurrence. As may be inferred, tumors of connective tissue origin may arise from any situation where the prerequisite parent cells occur. Fibromas, myxomas, and lipomas are most likely to arise from the integument, while simple tumors composed of cartilage or bone or a mixture of these two tissues may be expected to arise from situations where cartilage or bone normally occurs. Certain multipotent mesenchimal cells may and sometimes do give rise to cartilage and bone in situations where these tissues ordinarily are not expected. Of the connective tissue tumors, the group designated

histiocytic sarcoma is capable of the widest anatomic distribution. Situations in which this tumor has occurred include the wattle, esophagus, subcutis, liver, spleen, and ovary.

Osteogenic sarcomas may arise wherever periosteal tissue occurs.

Effects on the host. The malignant varieties of connective tissue tumors are potentially lethal, and the effect on the host depends on whether metastasis has occurred and what organs are affected. One gets the impression that these tumors, especially the fibrosarcomas and the histiocytic varieties, grow rapidly, and death or extreme debility may ensue relatively soon after the disease has become disseminated. Most of these tumors produce a progressive destructive disease that invariably results in death.

Gross and microscopic description. Like many other tumors, those derived from connective tissue elements seldom have sufficient distinguishing gross characteristics to make their identification certain. Fibromas are usually circumscribed, fleshy, nodular or oval masses that may be soft or firm (Fig. 32.1). A capsular covering usually can be recognized. Myxomas are likewise more or less circumscribed and of soft consistency, and the slimy mucinous

FIG. 32.1 — (1) Fibroma encircling the intestine. (2) Fibroma in the ventricular wall of the heart.

or gelatinous character of most specimens is evident. A lipoma, being composed largely of fat, offers little difficulty in recognition. The identifying features of benign tumors of cartilage or bone should be obvious.

Gross features that might enable one to distinguish malignant connective tissue tumors from other malignant growths are not to be relied on when an accurate diagnosis is desired. Although signs suggestive of malignancy, such as lack of encapsulation, multiplicity of lesions within the same general region, or the presence of contiguous implantations or distant metastatic growths, usually will permit no doubt as to the malignancy of the process, specific features that might enable one to recognize the specific character of such a neoplasm are missing. The diagnosis of these tumors, as of most others, must depend on microscopic examination.

Special features. One of the most interesting features of connective tissue tumors of the chicken is the transmissibility of some forms by filter-passing agents. The transmissibility of a considerable number has been studied, and a huge literature has accumulated which deals with these investigations (Claude and Murphy, 1933; Foulds, 1934). A variety of such agents has been studied. They differ from each other both in the specific form of tumor they produce and in certain serologic characteristics. The existence of ultramicroscopic tumor-producing agents suggests the possibility of epidemics of neoplasms. Carr (1944) reported no neoplastic disease in chicks hatched from hens that had recovered from the Rous No. 1 sarcoma. Yolk of eggs laid by the hens contained a substance which neutralized the action of the sarcoma agent. Previously Carr (1943) had demonstrated persistence of the neutralizing substances, in blood serum of fowls recovered for 1 to 2 years, which were believed due to latent virus still in the tissues of the hens. Duran-Reynals (1940) reported similar neutralizing substances believed to exist as natural antibodies in the blood of adult normal chickens. Kenzy and Neuzil (1953) found such neutralizing antibodies in 60 per cent of serums from flocks with a high incidence of lymphomatosis, but such antibodies could not be produced by artificial with transmissible or nontransmissible lymphoid tumor material (Kenzy, 1953). In another series, Kenzy et al. (1961) found a rather marked variation in the occurrence of tumors and prevalence of Rous virus neutralizing antibodies in different flocks. Usually a low level of tumors was related to a low level of antibodies. Duran-Reynals et al. (1953) found that chickens recovered from and subsequently resistant to a lymphoid tumor were susceptible to implants of Rous No. 1 tumor. A good review of the antibody against tumor virus and other inhibiting factors is given by Bryan (1958) and should be consulted by one interested in these aspects. The RIF factor of Rubin (1960) is regarded by him as a naturally occurring avian leukosis virus which will produce resistance against Rous sarcoma virus in cultures of chicken embryo tissue. Rubin et al. (1962) studied a flock with high incidence of lymphomatosis and found that one of every six hens had persistent RIF viremia and regularly infected their embryos. These congenitally infected birds had no detectable antibodies to RIF. Birds not infected through the egg became infected by contact with those having a viremia and in turn developed a transient viremia followed by formation of antibodies to RIF. An etiologic relation between certain fibrosarcomas and fowl leukosis is discussed in the section describing fowl leukosis. The situation described by Kenzy (1953), of a myxosarcoma giving rise to a transmissible lymphoid tumor with a long incubation period, is unique. The diminishing titer of Rous tumor-neutralizing substances with the serial passage of the lymphoid tumor in chickens suggests only a partial association of the lymphoid tumor and the factor causing such neutralization. Some of the transmissible connective tissue tumors of chickens have been reproduced experimentally in other species of fowl. Harris (1955) has shown that the

natural resistance of older turkeys to *Rous* sarcoma can be overcome if the turkeys are given normal chicken blood intravenously when they are hatched. This induces a state of immunological tolerance. Perhaps most spontaneous connective tissue tumors of the chicken might prove transmissible if all the conditions conducive to success could be supplied. Duran-Reynals (1946a) was more successful with transplantation and demonstration of cell-free agents of sarcomas occurring in chickens 5 to 10 months of age. Carcinogenic chemicals have produced connective tissue tumors in birds under proper experimental conditions. However, such tumors do not appear transmissible by cell-free agents (Murphy and Sturm, 1941a, b, Peacock, 1946). Peacock (1946) studied the histology of 15 transplantable, chemically induced sarcomas and 3 sarcomas that could be transmitted by cell-free agents. He states that slight but definitely recognizable differences exist between tumors induced by chemicals and those by cell-free agents.

The intensive research on agents of chicken tumors will continue to provide new facts. The vascular lesions produced in ducks by a variant of the Rous sarcoma agent (Duran-Reynals, 1950), the leukemic form of an endotheliona induced by X-ray (Oberling *et al.*, 1953), and the hepatitis observed in chicken embryos with a strain of the erythroblastosis agent (Atanasiu, 1956) indicate that they are not rigidly specific as to their action. The visualization of the agents with electron microscopy allows study of their relation to structures within cells (Epstein, 1957) and the observation of agglutination of viral particles by immune serum (Beard *et al.*, 1957).

Fibroma and fibrosarcoma. A fibroma in its simplest form consists of rather adult fibroblasts and narrow to wide strands of collagen disposed parallel to the longitudinal axis of the fibroblasts. The fibroblasts vary in size and number according to the rate of growth. Fibromas that progress slowly show the greatest degree of differentiation in that more collagen is produced, and the resultant structure contains relatively few cells in proportion to the collagen present. The less differentiated forms are more cellular and less firm owing to a relatively small amount of collagen. In some instances markedly edematous regions occur in which the fluid causes separation and retrogression of the collagen fibrils. The edema in these instances should not be confused with the mucinous product of myxomatous tumors. If infection has occurred because of erosions at the surface, various phases of inflammation and necrosis may be recognized.

The essential features of fibrosarcomas are the immaturity of the type cells and the aggressive and destructive behavior of the neoplastic process (Fig. 32.2). Large, irregularly arranged, hyperchromatic fibroblasts are abundant, and mitosis is common. Collagen is present in variable amounts but is less abundant than in fibroma. Stromal elements, frequently disposed as irregular septa, may occur, and small blood channels usually can be recognized. In rapidly growing specimens, small to extensive regions of necrosis are likely to occur. Occasionally, widespread edema is present, causing considerable separation of the fibroblastic components.

Myxoma and myxosarcoma. Myxomas consist of stellate or spindle-shaped cells surrounded by a homogeneous, slightly basophilic, mucinous matrix. Long cytoplasmic processes may extend from the stellate cells and become fused with the matrix and collagen fibrils. In the malignant form of myxoma the mucinous matrix is less abundant than in the benign form, and the type cells are proportionately more numerous and more immature. The histogenesis and structure of primary myxomatous tumors are closely related to those of the fibroblastic tumors, the essential difference being that in myxomatous tumors the fibroblastic cells are more specialized and are capable of producing
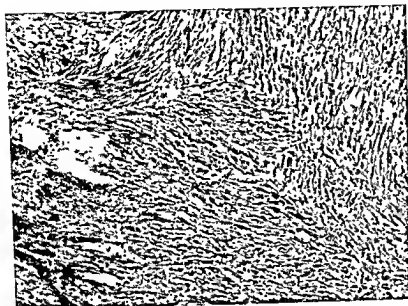
FIG. 32.2 — Fibrosarcoma in the musculature of the breast. The neoplastic process was bilateral and had metastasized to the lungs. ×120.

mucin in addition to the usual products such as fibroglia, collagen, and elastic fibrils.

Lipoma. Like other tumors of connective tissues, those derived from the lipoblast may be composed of adult cells and present the physical appearance of ordinary fat tissue, or the immature forms of the lipoblast may predominate, in which case the tumor presents a sarcomatous appearance. The microscopic appearance of lipoma is extremely simple. The process consists of compactly arranged, moderately large to extremely large polyhedral cells filled to capacity with a large fat globule or several small ones. The nucleus may be crowded to the periphery of the cell and be entirely obscure. The stroma consists of narrow strands of connective tissue which provide support for the blood vessels. A thin capsular covering is usually evident. Grossly the yellowish, fatty consistency of lipomas of the chicken strongly suggests their true nature. Frozen sections stained with scarlet red provide convincing evidence of their lipomatous character.

Osteoma and osteogenic sarcoma. The rarity of these forms of connective tissue tumors among chickens has precluded our obtaining examples for study. Heim (1931) cited the report of one case of osteoma in the chicken. The brief descriptions which follow represent a study of tumors of bone from animals other than fowls. The structure of an osteoma simulates bone with the exception that much of the finer histologic detail is lacking. The process consists of a diffusely disposed acidophilic matrix of osseomucin separated at irregular intervals by collections of osteoblasts. Lamellae may be recognized, and the mimicry may include structures comparable to or suggestive of haversian canals. Osteogenic sarcomas are usually very cellular, infiltrative growths which destroy the surrounding tissues and readily metastasize. The immaturity of the cells is usually evident, and mitotic figures are commonly numerous. The cells may be spindle-shaped, ovoid, or polyhedral, and foreign body giant cells occasionally occur. Although an osteogenic tumor is usually a highly cellular and frequently a rapidly growing tumor in which immature or undifferentiated cells may constitute the bulk of the structure, a few to many cells usually can be found in which

sufficient differentiation has occurred to produce osseomucin. The finding of this substance, which has a homogeneous acidophilic appearance, is usually sufficient to reveal the true character of these tumors.

Cbondroma and chondrosarcoma. Only a few cases of simple chondrogenic tumors in chickens have been reported (Heim, 1931). It should be kept in mind that cartilage cells are the product of specialized fibroblasts of mesodermal origin, and in the early phases of their functional differentiation these cells have the appearance of ordinary mesenchymal cells. By a gradual process of transition, adult cartilage cells finally are evolved which produce chondromucin in addition to collagen and elastic fibrils. The adult cartilage cell is the end phase of the transition process and is not capable of the production of other cartilage cells. These must arise from certain fibroblasts that have the latent capacity to produce chondromucin. Chondroma, the benign form of these tumors, is characterized microscopically by a typical and unique structure. It consists of groups of two or more cartilage cells lying in a homogenous matrix of chondromucin. The tumor may be separated into lobular compartments by strands of fibrous connective tissue. The appearance of the rapidly growing or sarcomatous variety of cartilaginous tumor is more subject to variation than is that of the benign form. In the former all gradations in the development of the type cell from the most immature phase to the fully adult cartilage cell may be seen in a single microscopic field. The undifferentiated cells are spindle-shaped, while those in the zone nearer the adult or fully differentiated cartilage cells are polymorphic.

Although, as we have mentioned previously, simple chondrogenic or osteogenic tumors occur rarely in chickens, connective tissue tumors, usually of the malignant type, occasionally occur in which cartilage or bone or both may be present. In these instances tumors that are primarily and predominantly fibrosarcomas in structure have within them areas of chondrogenic or osteogenic tissue. Tumors may be found composed of immature fibroblastic connective tissue, cartilage, and bone. Such specimens may be designated fibrochondroosteosarcoma. Tumors composed of immature chondrogenic and osteogenic tissues also have been noted in chickens. The multipotency of the primitive fibroblast accounts for these unusual neoplastic combinations.

Histiocytic sarcoma. This type of connective tissue tumor was first described adequately and established as a definite neoplastic entity by Jackson (1936a). Those interested in the histogenesis of histiocytic sarcoma as exemplified by the so called Rous sarcoma should consult McGowan (1928), whose views are, in general, in agreement with those of Jackson (1936a). Jackson reported having encountered 14 cases among 203 cases of neoplasia of poultry.

Perek (1960) reported an outbreak of histiocytic sarcomas in a flock of 600 one-year-old hens. During a four-month period, 200 died and about 200 had to be slaughtered. Tumors of the liver were found in 90 per cent though the exact number examined was not stated. Five of six birds carefully examined in the laboratory had tumors of the liver and in some the spleen, kidney, and skin were affected. The tumor was transplanted through 59 passages and the agent was filterable. This was suspected of being an infectious granuloma but bacteriological examinations were negative.

In our series of 11 cases the ages of the birds varied from 35 days to 16 months, with 7 of them aged 1 year or less. While it would appear that histiocytic sarcoma may arise from almost any situation in the body, the ovary seems to be one of the sites of predilection. Other sites in which histiocytic sarcoma has occurred include esophagus, wattle, breast, pectoral muscle, subcutis, liver, and spleen. Campbell (1943) described a case primary in the kidney. Other organs, such as the lungs, gizzard, and heart, also have been involved.

but involvement of these organs and of the serous tissues of the abdomen probably represents secondary rather than primary manifestations of the neoplasm.

The gross appearance of histiocytic sarcomas is not diagnostically characteristic. Encapsulation does not occur. These tumors are invasive and extend into the surrounding tissues in a rather diffuse manner. Those that arise in the ovary may be expected to extend to the contiguous tissues, especially the serosa, and to fuse the intestines, mesentery, and pancreas into a single unit. One should keep in mind, however, that similar spread by implantation is rather characteristic of other types of malignant ovarian neoplasia. Distant metastasis of histiocytic sarcoma to the lungs may occur, and occasionally microscopic foci may be found in the kidneys. The metastatic growths in the lungs are often severe, and both lungs may appear as solid masses of neoplastic tissue. All histiocytic sarcomas are potentially malignant.

Microscopically, to the inexperienced, histiocytic sarcomas are likely to present a structure of confusing complexity. These tumors usually appear as a mixture of two or more types of cells that, while morphologically dissimilar, are in fact closely related histogenetically (Fig. 32.3). The cells that may be recognized are (1) spindle-shaped cells that usually appear in groups or bundles somewhat like the structure of simple fibrosarcoma; (2) stellate reticulum-producing cells (fixed histiocytes); and (3) large phagocytic cells or macrophages (free histiocytes), the latter usually showing evidence of their functional specificity. In addition, one usually can observe numerous transitional forms, of which many are polymorphic.

Foreign body giant cells are quite often present in regions where macrophages are numerous. In those instances in which the tumor has metastasized, there is usually present in the primary situation a greater number of spindle-shaped cells than there are macrophages or histiocytic forms. In metastatic foci the reverse is usually true, and macrophages and the primitive histiocytic forms predominate.

The microscopic diagnosis of histiocytic sarcoma is facilitated if the varied character of the type cell is kept in mind. The cellular constituents vary from spindle-shaped elements resembling fibroblasts to
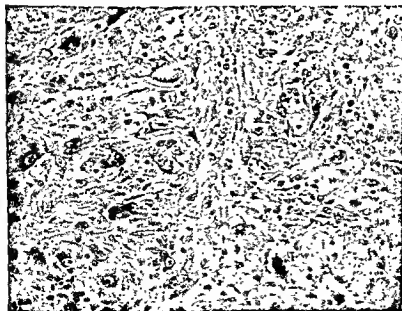
FIG. 32.3 — Histiocytic sarcoma of the spleen. The varied character of the cellular constituents is shown. ×285.

FIG. 32.4 — Neurogenic sarcoma apparently arising from the dorsal root ganglion of the first thoracic spinal nerve.
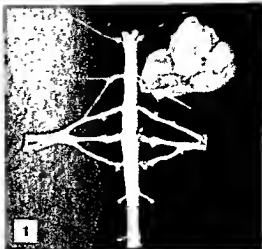
stellate and polygonal elements of bizarre form. A mixed type of structure is often the most striking feature of the microscopic picture. The recognition of the various constituents comprising these so-called mixed tumors is sufficient to distinguish their true character.

Neurogenic sarcoma. Neoplasia of connective tissue elements of peripheral nerve trunks has been reported by Jackson (1936a), Olson and Bullis, (1942), and Duran-Reynals (1946a). Jackson observed such tumors as multiple nodules usually associated with the cutaneous nerves. Olson and Bullis described five cases. In two cases the tumor was single, and involved the ganglia of the dorsal root of the brachial nerve plexus (Fig. 32.4); in one case the tumor was found in two widely separated nerve trunks; and in the remaining two cases the lesions were not localized within the nerve but infiltrated the tissues adjacent to the affected nerves.

The minute structure of the tumors was quite similar and consisted of fibroblastic elements of low malignancy which showed a distinct tendency to assume a whorl-like arrangement, sometimes with fissures The histogenesis of these tumors is rather obscure, but it seems probable that they may originate from the fibrous sheath of the nerves.

Proper classification of these tumors is difficult. The whorl-like formations are similar to multiple neurofibromatosis of man, but, except for Jackson's case, the localization and invasiveness of these tumors are dissimilar from the characteristics of neurofibromatosis in human beings. A palisading of nuclei often noted in neurogenic sarcoma of human beings was not observed in any of the cases in chickens.

Metastasis and malignancy. Since the type cell of the benign and malignant forms of the connective tissue tumors is fundamentally the same, all of these tumors are potentially malignant. However, it is recognized that many grow slowly, never invade the surrounding tissues, and continue indefinitely as strictly localized processes. It is our impression that in chickens the benign forms of connective tissue neoplasms occur much less frequently than those that are malignant. The malignant forms are capable of widespread metastasis with secondary foci occurring in the visceral organs. The most striking metastatic manifestations are those of the lungs and of the intestinal serosa. In the histiocytic sarcoma there is some evidence that what may appear to be secondary foci of metastatic origin are in fact independent tumors arising as a result of a process that is systemic rather than local.

Diagnostic characteristics. The features that aid in the diagnosis of the connective tissue tumors are those associated with derivatives of the mesenchyme. The recognition of intercellular fibrils, reticulum, collagen, mucin, cartilage, or bone will suggest the connective tissue origin of these tumors.

TUMORS OF MUSCLE TISSUE

Two general classes of tumors of muscle tissue are recognized. Tumors of one class, known as rhabdomyoblastomas, are composed of muscle cells that have the inherent ability to produce within the cytoplasm both longitudinal and cross striations. Tumors of the other class, designated leiomyoblastomas, are composed of smooth muscle cells and their associated elements. Tumors of either class may be benign or malignant. The benign form of rhabdomyoblastoma is known as rhabdomyoma, the malignant form as rhabdomyosarcoma. Benign leiomyoblastomas are designated leiomyomas, while those that are malignant are known as leiomyosarcomas.

A review of the literature indicates definitely that rhabdomyoblastoma is among the rarer forms of neoplasms of chickens. So far as we know, only a few cases have been reported. Meyer (Feldman, 1932) reported a rhabdomyoma that arose in the skeletal musculature of the sternum of a chicken. The tumor was multiple and consisted of six separate masses measuring from 1 to 2 cm. in diameter. They were composed largely of striated muscle fibers, partially separated by strands of connective tissue. On account of the mixed character of the growth, Meyer designated the tumor "fibromyoma striocellulare." Another case of rhabdomyoblastoma was that reported by Peyron and Blier (1927). The tumor arose in the region of the hip joint of a rooster, grew slowly, and proved transplantable. Babic (1931) described rhabdomyomas of the pectoral muscle and submental region in a chicken. Olson and Bullis (1942) found two cases of neoplasia

which were diagnosed rhabdomyoma. In one bird the tumors were multiple with a peculiar bilateral symmetry of occurrence. In the other case a small tumor was found in the semitendinosus muscle with a secondary nodule on the intestine. In the remarks that follow, tumors of smooth muscle only are considered.

Frequency of occurrence. Tumors composed of the elements of smooth muscle are among the commoner neoplasms of chickens. In Jackson's (1936a, p. 432) series of 43 primary tumors of the female reproductive system, 15 were leiomyomas. Among 384 neoplasms of chickens encountered by Olson and Bullis (1942), 84 were leiomyomas. Nelson (1946) found 59 cases in a breeding flock of 1,108 hens, all of which were subjected to necropsy.

Sites of occurrence. Although leiomyoblastomas may arise from smooth muscle wherever the tissue occurs, the vast majority of the tumors occur in the ligament of the oviduct or in the oviduct proper. Other situations in which tumors of smooth muscle have occurred include the muscular walls of the large and small intestines, the mesentery, the gizzard, and the crop.

An unusual case was described by Jackson (1936a, p. 532) in which a mass weighing approximately 1 kg. and designated leiomyoma fibrosum involved the ovary. Multiple tumors of similar character affected also the ovarian bursa and the proctodeum. In another case mentioned by Jackson, a leiomyoma involved the ovary and the oviductal ligament.

Effects on the host. Most tumors composed of smooth muscle apparently grow slowly and require considerable time to attain sufficient size to interfere seriously with the normal functioning of the involved or adjacent tissues. Since these tumors are seldom invasive or destructive locally, the ultimate effect, if any, that they may exert on the host will depend largely on the amount of mechanical interference their presence may have on proper functioning of the involved parts. Those of the oviduct and of the broad

FIG. 32.5 — A relatively small leiomyoma in the mesosalpinx.
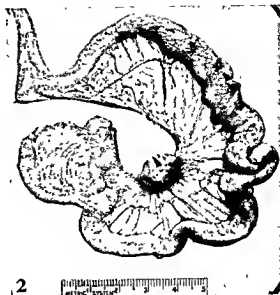
ligament could conceivably reduce or preclude egg production. Actually, Olson and Bullis found that 18 birds that had leiomyoma of the oviduct or the mesosalpinx were more than average in egg-laying ability. The interval between the last egg laid and necropsy varied from 1 to 75 days and averaged only 12 days. A causal relation between a long period of heavy egg production and development of leiomyoma of the mesosalpinx was suspected by Olson and Bullis. Nelson's (1946) data do not indicate a strong familial tendency for the disease. Leiomyomas of the intestines or other hollow organs might provide obstruction to the free passage of ingesta. If the tumor is malignant and secondary subserous implantations have occurred, ascites may develop.

Gross and microscopic description. Most leiomyoblastomas are smooth, elongated, ovoid, or irregularly spherical tumors. The benign forms usually are covered with a capsular structure that can be removed with difficulty. These tumors are resilient and of firm consistency. The size varies from 1 cm. or less to large masses several centimeters in diameter. They are flesh pink to grayish-white, and when they are cut across, the structure frequently appears distinctly fibrous. Although some specimens have a pedunculated form of attachment to the tissues from which they arise, in most instances these tumors are attached firmly by a rather broad base or over a considerable portion of their structures to the adjacent tissues (Fig. 32.5).

Microscopically, a typical leiomyoma presents a compactly knit structure composed of neoplastic smooth muscle cells. The degree of cellularity varies somewhat with the rate of growth, the cells being most numerous in those tumors in which the rate of growth is accelerated. Groups of cells usually are arranged in units of bundles which are disposed in every conceivable direction. The nuclei are ovoid or often elongated and contain a considerable amount of granular chromatin. The amount of connective tissue present between the respective bundles of muscle cells is subject to considerable variation. In some regions it is unrecognizable, while infrequently the fibrous elements may equal in amount the muscular tissue. Vascular channels are usually numerous.

In leiomyosarcoma the cells have the appearance of immaturity and are more numerous than in the slowly growing benign form. Cells undergoing mitosis are
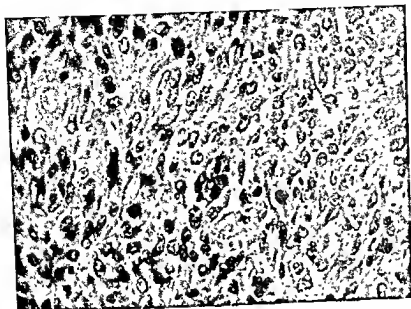
FIG. 32.6 — Malignant leiomyoblastoma (leiomyosarcoma) of the oviduct of a 2-year-old hen. ×660.

seen frequently, and the invasive tendencies of the process usually can be distinguished (Fig. 32.6).

Metastasis. As mentioned previously, tumors composed of smooth muscle cells are seldom malignant, and consequently metastasis is observed infrequently. Should metastasis take place, the liver and the lungs are the most likely sites of secondary foci.

Diagnostic characteristics. Tumors that arise in intimate association with tissues or organs containing smooth muscle are likely to be leiomyoblastomas. This is especially true of tumors of the wall of the oviduct or of the ventral ligament. The presence of smooth muscle cells arranged in interlacing strands or bundles disposed in a divergent manner is fairly characteristic. In differentiating tumors of smooth muscle from certain connective tissue tumors, the van Gieson stain may be helpful.

TUMORS OF BLOOD AND LYMPH CHANNELS

General considerations. Neoplasia may arise from the elements of the blood and the lymph vessels. Tumors developing from neoplastic growth of blood vascular channels are called hemangiomas, and those from lymph channels, lymphangiomas. Different types of growth may occur. In some instances the newly formed vessels are small and capillarylike, and the terms "capillary hemangioma" and "capillary lymphangioma" are applicable. In other tumors the blood or lymph spaces are large, and "cavernous hemangioma" or "cavernous lymphangioma" is the appropriate term. The tumors may be benign or malignant. In some hemangioblastomas, particularly those which are malignant, parts of the cellular mass may not show any evidence of differentiation and may appear similar to a fibroblastic sarcoma. The general character of such tumors is revealed, however, by the more differentiated regions in which distinct vascular channels are formed. Such tumors require careful study for correct identification.

Jackson (1936a, pp. 17–18) pointed out the difficulties in connection with the term "endothelioma," which sometimes is used in connection with tumors of vascular channels. The lumen of both normal and neoplastic vascular channels is lined with specialized cells called endothelial cells. Whether these are derived from the same

source as the angioblast forming the vessel wall or have an independent origin is a moot question. While some tumors have been designated simply as endotheliomas, their exact character, except in certain cases, is extremely vague. Such a diagnosis might refer to a tumor of the lining cells of blood channels. It might refer to a tumor of the meninges of the brain or spinal cord. It might also refer to a tumor arising from the reticulo-endothelium which is disseminated so widely in all organs of the body that the existence of such a specialized tissue is not often recognized. In actual practice, the term "endothelioma" usually can be avoided by careful study of the material, which enables one to arrive at a proper classification. Thus, meningeal tumors of this type are properly a form of fibroblastic neoplasia. Those of the reticulo-endothelial system are more properly associated with neoplasia of the hemopoietic organs. A tumor of the endothelium of vessels would be extremely difficult to recognize unless there was sufficient differentiation to form capillaries, in which case the origin of the cells would be in question and, furthermore, the tumor probably would be recognized as a capillary hemangioma.

Blood vascular tumors. *Frequency of occurrence.* Tumors of blood vascular tissue appear to be relatively infrequent in the chicken as judged by reports in the literature. Heim (1931) found 5 cases in the literature he reviewed. These included 4 cases of multiple hemangioma reported by Schurman and Pauly. The tumors affected the skin, musculature, mesentery, serosa of the duodenum, kidney, lungs, and subcutis of the throat. Darcel and Franks (1953) suggest that the disease of the skin may be more common than generally realized since it is not often the cause of death and it may occur as a familial angiomatoid lesion. Babic (1931) described 4 cases of hemangioma in chickens. These involved the liver in 2 birds and the skin and subcutis of the head and the eyelid in another chicken. In the fourth case the tumor was a cavernous hemangioma on

the peritoneum. Babic also described an angiomatous nevus of the wing in a canary. Olson and Bullis (1942) found 5 instances of hemangioma in a collection of 384 tumors of chickens. Three were of the cavernous type, and 2 were capillary hemangiomas. Only 2 of the tumors were considered malignant. The liver was affected in 4 of the 5 cases, suggesting a predilection of the liver of the chicken for the development of hemangiomas. In 2 cases the tumor in the liver was so small that it easily might have been overlooked. Ball (1915) reported a hemangio-endothelioma of the iris in a 21-week-old pullet.

Gross and microscopic description. Hemangioblastomas are variable in appearance according to the type of tumor. The large cavernous form is characterized by greatly distended blood spaces whose lining is a thin wall of endothelial cells (Fig. 32.7). The distended blood spaces often protrude from the affected organ or tissue. Such tumors are typical in macroscopic appearance. Capillary hemangiomas may appear as solid masses of neoplastic tissue varying from gray-pink to red. Histologically, their character is apparent. Small capillaries containing blood are the essential features. All gradations between the cavernous and capillary forms may occur. Hemangiomas of the skin are often multiple and may involve the underlying musculature (Monlux and Delaplane, 1952), and Rigdon (1954) has reported spontaneous regression of such tumors.

Lymph vascular tumors. Tumors of the lymph vascular elements may possess the same histologic features as those of the blood vascular system, except that lymph instead of blood is contained within the spaces formed by the tumor cells. A few blood cells may be found occasionally in the lymph. Macroscopically, milky fluid (lymph) may be recognized in the cyst-like structures of cavernous lymphangioma.

Very few cases of lymphangioma have been described in the chicken. Heim (1931) mentioned one case of lymphangioma of the eyelid in a bird which was found by Teutschlaender. Babic (1931)

FIG. 32.7 — Cavernous hemangio-endothelioma of the mesentery. ×70.

described a lymphangiolipoma of the mesenteric serosa in a chicken. Olson and Bullis (1942) encountered one case of lymphangioma in which the tumor was a pedunculated mass attached to the ovary.

Telangiectasis. Telangiectasis may be considered as a benign form of hemangioblastoma in which a group of blood or lymph vessels becomes dilated with blood or lymph. The pathogenesis of telangiectasis is not known, and different authors have suggested that it is either a congenital or a hereditary disease or that it follows injury and repair to an organ in which the circulation has been disturbed (Ewing, 1928; Feldman, 1932). Telangiectasis may be multiple and capillary or cavernous.

TUMORS OF HEMOBLASTIC ORIGIN

Lymphocytoma. Definition and terminology. The neoplastic lymphocyte is the type cell of a common tumor of chickens to which the simple descriptive term of lymphocytoma may be applied. Many other terms have been given to this tumor. These include round-cell sarcoma, lymphadenoma, lymphocytomatosis, lymphomatosis, lymphatic leukosis, leukoblastic leukosis, leukemia, and others. The question of terminology is made somewhat

difficult because of the frequent association of lymphocytoma with fowl paralysis and fowl leukosis. This has come about as a result of experiments intended to demonstrate the transmissibility of these diseases. The relationship between lymphocytoma and fowl paralysis is an unsettled question since research has yielded conflicting results. The report by Davis and Doyle (1947a) provides a concise review of the literature on this question and shows the diverse conclusions reached from attempts at experimental transmission experiments. Sevoian et al. (1962) reported an isolate from a naturally affected chicken that caused neural, ocular, and visceral diseases. This isolate produced lymphomatosis in 90 to 100 per cent of inoculated chickens within two to three weeks. For the present, etiologic considerations should not be allowed any part in the nomenclature of these diseases. We have adopted the precept of dealing only with the pathologic anatomy in assigning names to these conditions.

Histogenesis. The lymphocyte is a most important cell in the animal organism and has many diverse physiologic functions. Our knowledge of these functions is far from complete. The lymphocyte is an integral part of the widespread reticuloendothelial system. It is normally an un-

stable cell and may assume many different forms. Jordan (1936) expressed the belief that the lymphocyte is a hemoblast capable of developing into any type of blood cell. According to him the large lymphocyte, as found in the circulating blood, represents a young cell with the foregoing potentialities. The small lymphocyte is a more mature adult cell which has lost or outgrown the ability to become transformed into other types. The factor or factors which govern and direct the transformation of lymphocytes into other cells are not well understood.

The histogenesis of lymphocytoma is likewise obscure. The orthodox concept of neoplasia that a tumor begins as a result of a cell or a localized focus of cells assuming a state of neoplasia does not seem applicable in the case of lymphocytoma. Lymphocytoma appears to be more of a systemic disease in which the lymphocytes in widely scattered regions become transformed simultaneously into neoplastic cells. This feature of lymphocytoma suggests the existence of a specific stimulus which, when applied to lymphocytes in a susceptible stage, causes them to become capable of the unrestricted growth characteristic of autonomous proliferation. Lymphocytoma is found more often in certain organs rich in lymphoid tissue than in others. Although it commonly affects the liver, gonad, kidney, and spleen, other organs likewise rich in lymphoid tissue, such as the marrow, bursa of Fabricius, and thymus, are affected less commonly. This fact suggests that the lymphoid tissue in the latter group of organs is in a functional state different from that in the organs first mentioned, rendering it less susceptible to the hypothetical stimulus for neoplasia.

Lucas and Oakburg (1950), upon studying the lymphoid tissue of the pancreas, suggested that 1 per cent lymphoid tissue in the pancreas be considered evidence for lymphomatosis. They calculated that birds of their experimental material required 33 days to develop grossly visible tumors and that 90 per cent of the time

was involved in developing the 1 per cent area of lymphoid tissue, after which the process developed rapidly. More recently, Lucas et al. (1954) extended this work to include the liver and spleen of chickens which received a presumed lymphomatosis agent. A similar reaction was found in the liver and spleen but was less sustained in the liver. These workers have developed an intriguing hypothesis in which they suggest that lymphoid foci develop in response to an agent and that the infective stage be considered "lymphomatosis"; when the lymphoid foci become neoplastic and grossly visible the condition is in the neoplastic stage and should be called lymphocytoma. The results of future research should confirm or deny this interesting hypothesis.

Structurally, three forms of lymphocytoma may be recognized. These are the diffuse, the nodular, and the combined diffuse and nodular forms. In the diffuse form the affected tissues are infiltrated diffusely with neoplastic lymphocytes which crowd and replace the parenchymatous tissues of the involved organs. The nodular form is characterized by a follicular arrangement of the tumor cells, which are confined by a more or less well-developed retaining wall of connective tissue, rich in reticulum. The third form of lymphocytoma is a combination of the diffuse and the nodular forms. In such cases a single organ may show both forms, or one organ may be affected with one form and other organs with the other form.

A possible explanation has been suggested for the existence of these diverse forms of lymphocytoma (Olson and Bullis, 1942) based on the hypothesis that they develop because of the inherent resistance on the part of the individual host to the growth of the tumor. Thus diffuse lymphocytoma develops in birds that have little resistance to the growth of the tumor, and nodular lymphocytoma develops in birds which are able to muster considerable resistance. The third form of combined diffuse and nodular lymphocytoma develops in birds that have only a moder-

ate degree of resistance to growth of the tumor. Evidence in support of this hypothesis is provided by several facts. Involvement of fewer organs and less extensive damage usually are noted in the nodular form of disease than in the diffuse form. Birds affected with the nodular form are often emaciated, suggesting a prolonged course, whereas the carcass in cases of the diffuse form is usually well nourished. The response of connective tissue in the nodular form suggests an attempt to limit the growth of the tumor.

Solution of the problem of the causation of lymphocytoma is an almost essential requirement for developing a sound conception of the histogenesis of the disease. For the present it may be said that lymphocytoma develops from lymphoid cells which are scattered widely throughout the body, but the stimulus or mechanism by which the cells become malignant is yet unknown.

Frequency. Lymphocytoma is without question the most common form of neoplasia affecting the domestic chicken. Olson and Bullis (1942) found 213 cases of lymphocytoma in a collection of 384 tumors, an incidence of 55.5 per cent. It has been recognized by poultry pathologists that lymphocytomas may be much more common in some flocks than in others. They may be as common as to constitute a serious economic problem in some flocks. Experimental results such as were reported by Hutt et al. (1941) suggest that the incidence of neoplasia can be controlled by selective breeding. Since they stated that "approximately 95 per cent of the deaths attributed to neoplasms were caused by lymphomatosis of one kind or another," it is inferred that by selective breeding lymphocytoma would be affected significantly. The exact effect of such a breeding program on the incidence of lymphocytoma alone is unfortunately not available, since suitable data on which to draw conclusions are not given.

A summary report of Hutt and Cole (1947) indicates a marked difference of incidence in the pathological manifestations classified by these workers as "neoplasms" in two lines of chickens selected for resistance and the susceptible line of chickens developed by them. While their classification of neoplasms includes all varieties of neoplasia and the so-called neural and ocular forms of lymphomatosis, the condition designated lymphocytoma is probably affected by such a breeding program since it is so commonly encountered in chickens. Another factor has been woven into the fabric of the thesis on "resistant" and "susceptible" strains. This is the question of exposure to the as yet undefined "agent of lymphomatosis" which was given impetus by the reports of Barber (1942, 1943) and considered as a factor in the work reported by Waters (1947). Waters (1945, 1954) believed transmission through the egg was indicated by data which he studied. Cole and Hutt (1951) came to the opposite conclusion. Burmester and Waters (1955, 1956) believe that hens which shed the agent in their eggs are important in creating a few "carrier" chicks which spread the disease to other chicks during the brooding period. There is also some evidence to suggest that parental immunity may also play a part in this process (Burmester et al., 1957).

The disease usually affects chickens less than a year old. Birds that had lymphocytoma in the series of cases studied by Olson and Bullis had an average age of about 8½ months. The youngest bird affected was 6 weeks old and the oldest was 2 years of age. It was formerly believed that males were affected about as often as females when one considered the disproportionate numbers of male and of female chickens in the general poultry population. The data of Olson and Bullis tend to suggest that the male is affected with lymphocytoma less commonly than is the female. Burmester (1915) reported that the incidence of lymphomatosis (presumed to include lymphocytoma, neurolymphomatosis, and the iritis commonly associated with neurolymphomatosis) was twice as great in female as compared to male

uninoculated chickens. Marine and Rosen (1910, 1941) noted a rather high incidence of lymphomatosis (which in most of their cases appears to be similar to lymphocytoma) in castrated male chickens. They suggested that an imbalance of hormones may have activated a latent tumor-producing agent in these birds. A higher incidence of lymphomatosis in castrated male chickens was also observed by Burmester and Nelson (1915) whose studies showed a similar effect on castrated females. They further reported that administration of a female sex hormone (diethylstilbestrol) lowered the incidence in capons but had no effect on intact males. Administration of a male sex hormone (testosterone propionate) seemed to lower the incidence of lymphomatosis in both males and females. Burmester and Nelson (1915) suggest that these hormones increase the resistance of the bird to lymphomatosis. Davis and Doyle (1947a) also reported a higher incidence of visceral lymphomatosis in females and capons than in intact male chickens.

Anatomic situation. Lymphocytoma may affect nearly every organ or tissue in the chicken, and in a given case usually more than one organ or tissue is affected.

In a series of 213 cases of lymphorytoma (Olson and Bullis, 1942) there were only 31 instances in which the disease was confined to a single organ or tissue. The liver, spleen, kidney, and gonad were the organs most commonly found to be affected. Nineteen different organs or tissues, exclusive of nerves and the circulating blood, were found to be involved with the disease. In this series the different combinations of organs or tissues that might be affected with lymphocytoma were studied. The great variation of the manner in which lymphocytoma may express itself is indicated by the fact that 152 different combinations were found. The more frequent of these combinations were as follows: ovary alone (10 cases); liver, spleen, kidney, gonad, and marrow (7 cases); liver, spleen, and kidney (7 cases); liver and spleen (5 cases); peritoneum alone (5

cases). The histologic form of the disease appears to be a determining factor with respect to the number of organs or tissues involved. The diffuse and the combined nodular and diffuse forms tend to be more widespread and affect more organs than does the nodular form.

In view of the tendency of lymphorytoma to become widely disseminated, it is logical to assume that nerve tissue may be affected with the disease. In 83 of the 213 cases of lymphocytoma studied by Olson and Bullis (1942), deposits of neoplastic lymphoid tissue were found in the peripheral nerves. The amount of tumor tissue varied from a localized, lightly infiltrated region to complete replacement of the nerve tissue and marked enlargement of the affected nerve. The presence of such deposits of lymphoid tissue associated with peripheral nerves raises the question whether they represent foci of lymphocytoma or are an expression of the disease known as fowl paralysis. The lesions of the nerves in fowl paralysis have been described as either inflammatory or neoplastic. When inflammatory, the lesions consist of polyblastic infiltration (lymphocytes, histiocytes, and plasma cells) often associated with the proliferation of the cells of the sheath of Schwann and degeneration of neurons in the ganglia. The neoplastic lesions differ from the inflammatory lesions in that the infiltrating lymphoid cells have a definitely neoplastic character, are multiplying actively, and may be so aggressive as to replace almost entirely the nerve elements within the sheath. The neoplastic process may penetrate the nerve sheath and infiltrate the adjacent surrounding tissue. Separation of the lesions into two such groups is complicated by those cases in which both types of lesions are found. Wight (1962) classified three histological types of lesions in 100 cases of fowl paralysis. Marked neoplastic changes were present in only 11 cases. Edema was the predominant change in affected nerves of an inbred stock of Brown Leghorns and infiltration with lymphocyte and plasma cells

the principal change in cases from several other sources.

Involvement of the nerves in some cases of lymphocytoma is obviously due to the penetration of the nerve sheath from without by neoplastic lymphoid tissue. However, those instances of lymphocytoma in which neoplastic lymphoid tissue is found in nerves at a site removed from other tissues affected with lymphocytoma constitute a difficult problem in interpretation. They may represent metastasis of the tumor from a primary focus situated elsewhere. The tumors may have developed in the nerve in response to a hypothetical causative agent of lymphocytoma. They may be lesions of fowl paralysis existing coincidentally with lymphocytoma. On the other hand, one may assume that a single agent is responsible for both fowl paralysis and lymphocytoma and that the type of response to the agent depends on factors as yet unknown. A final solution of this question must await the solution of the problem of causation.

Effects on the host. Lymphocytoma is usually considered to be a fatal disease. While this may be generally true, an affected bird may recover. In our material two such cases have been noted in which the birds had multiple tumors of the skin. Diagnosis of the disease was made by biopsy and histologic examination of representative cutaneous lesions. The birds were held under observation for several weeks, and the remaining tumors of the skin disappeared. A similar regression of a tumor in a visceral organ can occur. Davis and Doyle (1947b) have reported a study of monthly liver biopsies on 96 chickens done over a 10-month period. Some of the birds had been inoculated with material from a case of spontaneous visceral lymphomatosis, and others were uninoculated controls. These interesting data showed that fatal cases of the disease developed very rapidly. For example, biopsy material was normal in birds that died with lymphomatosis 3 to 4 weeks after biopsy. In some instances, lesions of the liver characteristic of lymphomatosis

were observed in biopsy material, and the lesions later disappeared, indicating recovery from the disease. St. Louis encephalitis virus has an oncolytic action on a transplantable lymphoid tumor in which the agent can multiply in the tumor but does not destroy cells. Changes do occur which are believed either to render the cells more susceptible to phagocytosis or to increase their ability to stimulate the defense mechanism of the host (Love and Sharpless, 1954).

There are no specific or pathognomonic symptoms displayed by birds affected with lymphocytoma. In many instances birds under relatively close observation may die from the disease without indication of ill health. In most cases, signs of a general disturbance of physical health are evident for a variable period preceding death. These signs are listlessness, inappetence, ruffling of the feathers, and general depression. An affected bird is often first noted to be standing in droopy attitude with its eyes closed and with an intermittent shaking of the head as though the sensorium were befogged. The location of the lesions may provoke distinctive signs referable to their situation. For example, tumors of the skin or musculature cause localized swelling. Involvement of the digestive tract may cause either diarrhea or obstipation, and involvement of a nerve may cause paralysis of the part supplied by the nerve. Palpation of the abdomen may reveal displacement of the viscera due to an enlarged liver. Emaciation will develop in cases of long standing, which are usually due to the nodular form of lymphocytoma.

Olson and Bullis (1942) obtained data on the egg production of 15 birds that died of lymphocytoma. These birds were considered average to slightly better than average producers of eggs. The rather rapid course of lymphocytoma is suggested by the finding of a relatively short interval between cessation of egg production and the death of the birds. This period averaged 38 days and varied from 4 to 73 days.

Olson and Dukes (1938) found that

the basal metabolic rates of two chickens affected with lymphocytoma were greatly increased over the normal level. In this respect these cases of lymphocytoma of the chicken were similar to neoplastic diseases of the lymphoid cell system as encountered in human beings. The rate of basal metabolism should be studied in more cases of the disease, and if it is found that an increase is a constant feature, the result might possibly explain the rapid wasting and emaciation associated with the more chronic form of lymphocytoma.

Gross and microscopic description. Organs or tissues affected with lymphocytoma have a gross appearance which varies with the extent of infiltration and the character of the process. Organs, which on gross examination appear normal, may contain neoplastic foci when examined microscopically. The characteristic color of neoplastic lymphoid tissue is gray-white, and the tissue may have a red tint in the more highly vascular areas. Necrosis of the tumor substance is not observed commonly but may develop in regions of the tumor in which the blood supply has been reduced by occlusion of the vessels either from pressure or from the infiltrative growth of the neoplasm.

As mentioned previously, the growth may be diffuse or nodular or a combination of the two. In diffuse lymphocytoma the affected organs are enlarged uniformly, and the color of the organs may change until it resembles the gray-white of the tumor. The extent of enlargement and the degree of change of color depend on the amount of tumor tissue present. Organs severely affected and in which there is much replacement of the parenchyma are quite soft. In nodular lymphocytoma the neoplastic tissue has a well-developed supporting framework of connective tissue which adds much to the firmness of the tumor. Affected organs contain nodular gray-white masses whose margins are discrete and sharply defined. The nodules may almost completely replace the parenchyma of an organ, reducing the latter to narrow bands compressed between the masses of tumor (Fig. 32.8). In some cases of nodular lymphocytoma, the tumor may be distributed throughout the affected organ and resemble the diffuse form of the disease. Such cases can be recognized by the firmer consistency of the tumor and its histologic appearance. Fairly frequently the tumor may erode the walls of the blood vessels and cause hemorrhage. Fatal hemorrhage into the peritoneal cavity may occur from

FIG. 32.8 — Lymphocytoma; nodular type, showing multiple lesions in the liver.
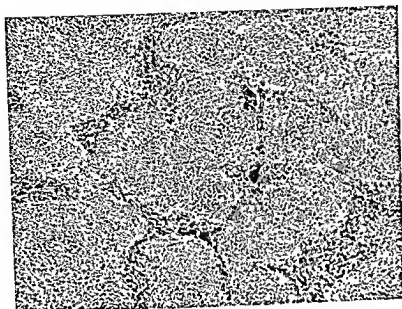
FIG. 32.9 — Nodular type of lymphocytoma, liver of a chicken. The multiple aggregates of neoplastic cells have characteristically replaced most of the parenchyma, leaving thin strands of hepatic cells and vascular channels. ×25.

rupture of the taut capsule of organs greatly enlarged from growth of the neoplasms. Such hemorrhages are noted most often from the liver and spleen.

Microscopically, the tumor consists of masses of proliferating neoplastic lymphoid cells situated extravascularly (Fig. 32.9). The foci of cells tend to develop most rapidly in the immediate vicinity of blood vessels. The cells of the tumor are quite uniform and comparable in size to the large lymphocyte or monocyte of the circulating blood. They tend to be spherical, although in the denser parts of the tumor they are so compact that the shape either cannot be distinguished or is distorted. The cytoplasm is relatively scant, is without specific granulation, and stains faintly blue with hematoxylin and eosin. The nucleus is relatively large and has a vesicular appearance. The chromatin is arranged as an irregular band at the nuclear margin and in small clumps in the nucleoplasm. One or two distinct nucleoli are usually present. Mitotic figures are commonly found. A fine meshwork of reticulum enclosing small groups of tumor cells can be demonstrated by appropriate histologic procedures.

In the diffuse form of lymphocytoma, the infiltration of the tumor between the parenchymatous cells of an affected organ appears to proceed without the slightest restraint. As the disease progresses, the tumor cells destroy and replace the normal cells of the organ. This process may continue until the affected organ is almost completely converted into a solid mass of tumorous tissue.

In nodular lymphocytoma a marked response of connective tissue accompanies the proliferation of neoplastic lymphoid cells. The connective tissue surrounds and isolates clumps of tumor cells, forming a sort of capsular wall. These foci of tumor cells may be small and isolated, but more often they are contiguous to other such foci. Sometimes several such foci seem to merge with one another, forming larger masses surrounded by a thicker wall of connective tissue. The fibroblastic components of the connective tissue wall are not anaplastic and would seem to be the response of the host attempting to delimit the growth of the tumor rather than a part of the tumor itself. Cases of lymphocytoma occur in which both the nodular and the diffuse form of the disease may be found in either the same organ or different organs of the same animal.

Occasionally, the cells of lymphocytoma

may either erode or infiltrate into the lumina of blood vessels and thereby enter the circulation in numbers sufficient to be mistaken for a leukemic state, but this process is distinctly different from that leading to true leukemia. It should be regarded rather as an embolic phenomenon.

In addition to such emboli of tumor cells, changes of the blood picture may be found occasionally in lymphocytoma. These are of a secondary nature. Foci of lymphocytoma are found fairly frequently in the bone marrow where they may disrupt normal hemopoiesis by mechanical means. For example, they may replace sufficient myeloid tissue to lead to a state of insufficiency, causing anemia and leukopenia. They may also mechanically dislodge unripe cells from the marrow, forcing them into the circulation.

The cellular changes of the circulating blood in birds suffering from lymphocytoma have not received adequate attention. Part of this neglect is due to the difficulty of detecting cases sufficiently early so that the changes might be studied during the development of the disease. Several authors have made blood smear examinations of such cases at varying intervals preceding death, but their main objective was a search for pathologic cells in the blood rather than a study of variations of the cells normally present.

A fairly complete study was made of the blood in one of our cases, a lymphoid tumor which later was demonstrated to be transplantable (Olson, 1941). Although in this case the tumor has not been designated as lymphocytoma because of its transplantable nature, other features are such as would cause it to be considered as a lymphocytoma. These changes in the blood were observed during the 25-day period preceding death of the bird and may be summarized briefly as follows: Erythrocytes, hemoglobin, thrombocytes, eosinophils, and basophils were only slightly affected. The number of heterophils, lymphocytes, and monocytes fluctuated widely and approached normal levels only near the terminal stage of the dis-

ease. The severity of the involvement of the bone marrow did not seem sufficient to explain the variations observed. It seems probable that the disease in this instance was associated with the production of noxious materials which were responsible for the increase of the numbers of heterophils, lymphocytes, and monocytes.

Extension. The aggressive nature of lymphocytoma is revealed in many cases by the extensive lesions in markedly enlarged organs. As stated previously in the section on histogenesis, lymphocytoma is probably a systemic disease in which the process is initiated in several sites at the same time. Extension of the disease by direct spread from one tissue to another is illustrated by those cases in which the peritoneum is involved. The peritoneum may become affected by extension of the disease from the ovary, and the intestine may be involved in turn from the previously affected peritoneum. Further examples of such extension of the disease require only examination and study of material that comes to necropsy. Although emboli of tumor cells may be demonstrable in the blood vessels, acceptable evidence of true metastasis is difficult to obtain.

In this connection it is pertinent to mention a few unsuccessful attempts at autoplastic transplantation of lymphocytoma. In these experiments (Olson and Dukes, 1938) bits of skin tumor were transplanted into the subcutis and musculature of the birds from which the tissue was obtained for biopsy. Although only a few trials were made, in each case the implants failed to develop despite continued growth of the original skin tumors. Failure of such autotransplants may be due to concomitant immunity such as displayed by a transplantable lymphoid tumor in which the immunity developed 10 to 15 days after the initial graft protected the bird against subsequent grafts even though the original graft continued to grow (Olson, 1915). These experiments bear on the question of metastasis for they suggest that metastasis may be of rare occurrence in lymphocytoma. This would

lead to the conception that extension of the disease is largely a matter of direct spread from one organ or tissue to another rather than of circulatory metastasis.

Diagnostic characteristics. With an adequate knowledge of the fundamental pathologic changes, it is usually a relatively simple task to differentiate lymphocytoma from other diseases. Myelocytoma, leukosis, fibrosarcoma, epithelioblastoma, and some types of granulomatous processes are conditions which should be considered in arriving at a differential diagnosis of lymphocytoma. General features which may serve as a guide in distinguishing between these diseases are set forth in Table 32.3. Other less common tumors may cause confusion in the differential diagnosis of lymphocytoma; histiocytic sarcoma is a good example.

Special features. In the older literature, one will encounter the term "round cell sarcoma" with surprising regularity. While such a term is descriptive of the shape of cells found in such cases, it does not give any information on the histogenesis of the tumor. Heim (1931) made a thorough review of the literature on neoplasia of the chicken and, in addition to discussing "round cell sarcoma" of the connective tissue, devoted another section of his report to "round cell tumors of unknown genesis." This latter group was subdivided further into a "large celled" form and a "small celled" form. No doubt examples of what we today call lymphocytoma were included in both categories, as well as histiocytic sarcoma and perhaps other types of tumor.

The relation between spontaneous lymphocytoma and experimentally transmissible neoplasms of lymphoid cells is not well understood. Furth (1935) expressed the belief that the disease produced by the "Strain 2" tumor-producing agent studied by him is rare in a spontaneous disease of chickens and dissimilar from the commonly occurring lymphocytoma. Both Pentimalli (1941) and Olson (1941) have found a spontaneous lymphoid tumor that was transplantable to experimen-

tal chickens. The original cases of both possessed features which would permit them to be classified as lymphocytoma, and the principal reason for not doing so was that transplantability had not been demonstrated as a characteristic feature of lymphocytoma (Olson, 1940, 1942, 1947; Engelbreth-Holm, 1942; Duran-Reynals, 1946b).

Burmester and Prickett (1945) described strains of transplantable lymphoid tumors similar and apparently immunologically related (Burmester and Belding, 1947) to the lymphoid tumor reported by Olson (1941). Burmester (1947) reports variation in the reaction of chickens to cell-free material from birds bearing different transplantable strains of lymphoid tumor. Brewer and Brownstein (1946) state that "lymphomatous" liver and spleen material from several birds produced visceral lymphomatosis in young chicks. Details are given on only one strain which was infective with fresh affected tissues by feeding and simultaneous instillation in the eye and nose as well as subcutaneous inoculation. This tumor does not appear to be characterized by a definite rate of growth. Davis and Doyle (1947a) describe transmission of visceral lymphomatosis with a slower rate of growth than that observed with the lymphoid tumor by Olson. These transplantable lymphoid tumors and cases of visceral lymphomatosis had no predilection for growth in nerve tissue.

Burmester et al. (1947, 1957) reported that visceral lymphomatosis and osteopetrosis could be induced in chickens by filtered material from a lymphoid tumor (RPL 12) which, when maintained by tissue transplantation, caused only lymphoid tumors (Olson, 1941). Similar results were obtained with filtrates from some, but not all, of the transmissible lymphomatous tumors originating in chickens of the laboratory flock (Burmester, 1947). In addition, visceral lymphomatosis occurred in chickens which received cell-free inoculums prepared from normal-appearing liver of embryonating

TABLE 32.3

COMPARISON OF LYMPHOCYTOMA WITH OTHER DISEASES

	Lymphocytoma	Myelomatoma	Leukemia	Fibrosarcoma	Epithelioblastoma	Granuloma
Age (average)	9 months	9 months	11 months	10 months	12 months or more	Any age
Frequency	Common	Uncommon	Uncommon	Uncommon	Rare	Common
Course	Acute	Acute	Protracted	Variable	Variable	Variable
Location	Liver, gonad, kidney, and other tissues	Peritoneum, liver, spleen, gonad, marrow, and other tissues	Bone marrow, liver, spleen, kidney. Recent hemorrhages may be present in fascia and intestinal mucosa	Any organ	Any epithelial tissue	Any tissue
Extent	Widespread or limited	Widespread or limited	Limited	Limited	Limited	Limited
Character	Nodular or diffuse	Diffuse	Diffuse	Localized	Localized	Localized
Texture	Soft or firm	Soft	Soft	Quite firm	Quite firm	Soft or firm
Color	Gray-white	Dull white	Organs pale	Gray-yellow	Not consistent	Yellow (necrosis common)
Blood	Usually normal, may show anemia, sometimes embolism of tumor cells	Anemia, myelocytes in circulation	Severe anemia, immature blood cells	Essentially normal	Normal	Sometimes leukocytosis
Histologic characteristics	Extravascular infiltration with neoplastic lymphocytes	Extravascular infiltration of neoplastic myelocytes. Tumor cells may appear in blood stream	Intravascular collections of neoplastic unripe blood cells	Infiltration with neoplastic fibroblasts	Masses of neoplastic cells of epithelial origin	Inflammatory reaction
Causative factors	Probably filterable agents	Unknown	Filterable agent	Unknown and filterable agents	Unknown	Trauma, pathogenic bacteria, fungi, parasites, degenerative processes

eggs, incubator debris, washings from the oral cavity and respiratory tract, and extracts of feces obtained from chickens of the laboratory flock (Burmester *et al.*, 1955; Burmester and Waters, 1955; Burmester, 1956). The disease also developed in chickens reared in direct contact with penmates that had been inoculated with the filtered material from Strain RPL 12. Burmester (1952) recognized several pathologic entities in the inoculated chickens. One response was death from visceral lymphomatosis, after a latent period of more than 3 to 4 months, characterized by an extravascular accumulation of neoplastic lymphoid elements in one or more visceral organs. Another response was the *occurrence of an intravascular process* with features resembling erythroblastic leukosis, and a few chickens had in addition fibrosarcomas and hemangioendotheliomas (Burmester, 1952). The intravascular process became more common after the causative agent or agents had been propagated in chickens for several generations as cell-free material, and now Burmester *et al.* (1959) recognize the process as erythroblastic leukosis. Olson and Rountree (1957) inoculated chickens with filtrate of Strain RPL 12 (obtained from Burmester) and only erythroblastic leukosis developed in the 15 weeks of the experiment. In addition, osteopetrosis occurred in chickens designated Line 15, obtained from Burmester, but not in an unrelated stock also in the experiment. The erythroblastic leukosis was characterized by neoplasia of the more immature red cells in the sinusoids of the marrow with leukostasis in the liver and spleen. Anemia was not marked, and death was probably the result of hepatic insufficiency. Burmester and Gentry (1956) reported that large doses of cell-free lymphomatosis material resulted in a high proportion of the intravascular syndrome (erythroblastic leukosis) which caused death early; whereas, low doses resulted in a high proportion of the extravascular lymphomatosis with death generally occurring after 4 months. It was suggested (Burmester,

1952) that the former may be the acute and the latter the chronic expression of the same disease, although the possibility that they were caused by different viruses was also pointed out. Burmester and Walter (1961) obtained some cases of visceral lymphomatosis in their Line 15 I chickens inoculated with Rous sarcoma virus indicating its relation to the disease. Rubin and Vogt (1962) obtained an avian leukosis virus from stocks of Rous sarcoma virus which interferes with infection and clone formation of Rous virus in tissue cultures.

The association of spontaneous lymphocytoma with fowl paralysis is a feature still deserving of special attention. Some comments concerning this question have been made in this section, and other comments may be found in the chapter entitled "The Avian Leukosis Complex."

Myelocytoma. The term "myelocytoma" was first applied to this disease by Pentimalli (1915) who described two cases. Ellermann (1920) recognized the condition in the course of his work with leukosis and called the disease aleukemic myelosis. Mathews (1929b) gave an excellent description of the disease under the term "leukochloroma." Since the myelocyte is *the type cell of the tumor, the term* "myelocytoma" seems fitting. Myelocytoma is a neoplastic disease of myelocytes and *may affect almost any tissue in the body.*

Histogenesis. The myelocyte may be recognized readily as the type cell of myelocytoma, but the source of the tumor cells is an unsettled question. Cells which are morphologically similar to those of myelocytoma *may be found in the bone marrow* and in foci of extramedullary myelopoiesis of normal chickens. These represent normal metamyelocytes and myelocytes, which are immature acidophilic granulocytes. Two types of acidophilic granulocytes are found in the blood and the hemopoietic organs of the chicken. The more numerous are called heterophilic leukocytes and fulfill a function similar to that of heterophilic or neutrophilic leukocytes of mammals. The other type is the true eosinophilic leukocyte of

mammals. Although ordinarily the adult heterophilic leukocyte of the chicken contains in its cytoplasm spindle-shaped acidophilic granules, the same granules are spherical at certain stages of their development. The granules of the eosinophilic leukocyte are likewise spherical in the early stages of their development and remain so in the adult cell. Thus it is obvious that while the two types of leukocytes may be differentiated readily by the shape of the granules when adult in form, there are no reliable criteria for distinguishing between these cells in their immature stages of development. Although there are other features by which the two forms of adult cells may be separated, these features do not become apparent except in the later stages of development and are lacking in the type cell of myelocytoma.

During embryonal life, the mesenchyme in parts of the body other than the bone marrow acts as a hemopoietic tissue. This function subsides and at the time of hatching and afterward is almost entirely taken over by the bone marrow. The ability of tissue other than bone marrow to produce myelocytes is not entirely lost in postnatal existence. Foci of such cells developing in the periportal regions of the liver and in the thymus are encountered fairly frequently. The cells of a myelocytoma may arise from any or all of these potential sources.

The tendency for myelocytoma to involve many tissues or organs makes it difficult to determine whether the disease arises from a single primary focus or is an expression of a systemic disturbance. Mathews expressed the opinion that the disease was primary in the bone marrow and metastasized from there to the other sites in which the tumor was found. This opinion is hardly tenable in view of the possibility for the origin of myelocytoma in other tissues where a potential source of myelocytes exists.

Frequency. Mathews (1929b) mentioned two flocks of chickens in which myelocytoma appeared as an enzootic. In one flock the losses from the disease were estimated to be 20 per cent and in the other flock 10 per cent. Although the flocks were small and not all birds that died were examined, this tendency of myelocytoma was well illustrated. Usually, however, myelocytoma is a sporadic disease in a flock. Thirty-six of the cases collected by Mathews were found during necropsy of 3,938 birds, an incidence of 0.91 per cent. Olson and Bullis (1942) found 17 cases of the disease during the examination of 2,304 birds, an incidence of 0.74 per cent.

The age of birds affected with myelocytoma is usually less than 1 year. Mathews (1929b) found most of his cases during the winter months of November, December, and January but expressed the belief that the factor of age was responsible for this apparent seasonal effect. Olson and Bullis (1942) found the incidence of myelocytoma in each of the 4 quarters of the year to be the same when the factor of age was considered.

Mathews noted the disease to be common in chickens of the Barred Plymouth Rock breed. The data of Olson and Bullis suggest a greater frequency of myelocytoma in Barred Plymouth Rock chickens than in Rhode Island Red birds.

Anatomic situations. Study of the distribution of lesions of myelocytoma indicates that nearly any tissue or organ of the body may be affected with the tumor. A notable feature is the tendency for myelocytomas to develop on the surface of bones in intimate association with the periosteum. These may be sheetlike or nodular masses and frequently assume a peculiar, bilaterally symmetrical aspect. Such bilaterally symmetrical deposits often are found affecting the periosteum of the ventral portion of the keel bone and of the ribs. There seems to be a predisposition for the tumor to collect near cartilage at the costochondral junctions of the ribs and about the annular cartilaginous rings of the trachea. In some cases the tumor is disposed irregularly about the bodies of the vertebrae, especially in the lumbo-sacral region. Oberling and Guérin

(1934b) described four such cases. Mathews found similar cases in which the tumor had infiltrated the bone, caused pressure on the spinal cord, and led to paralytic symptoms of transverse myelitis. Although myelocytoma tends to develop on the surface of the keel bones, ribs, vertebrae, and sometimes the flat bones of the skull, a similar involvement of the long bones of the legs and wings is infrequent.

The liver, spleen, ovary, and bone marrow, in addition to subperiosteal tissues, are affected with the neoplasm in most cases. Other organs and tissues are involved less frequently, although there is perhaps no tissue or organ which may be regarded as resistant to invasion by the tumor.

Effects on the host. Chickens coming to necropsy with myelocytoma are usually in a fair state of nutrition, suggesting either that the course of the disease is relatively rapid or that the disease has but little effect on the host. The former would appear more logical since the tumor itself has a distinctly malignant character and appears capable of rapid growth. In a few cases in which data on egg production were available, the period between cessation of egg production and death was short (average of 21 days in three cases). Mathews mentioned that the clinical symptoms of a slight indisposition observed in most cases did not exist for more than a week preceding death and that sudden death without noticeable symptoms sometimes occurred. Mathews also observed symptoms of transverse myelitis in two cases.

Relatively rare cases occur in which the tumor masses can be detected on examination of the exterior of the bird either about the head or about the sternum.

Gross and microscopic description. Myelocytoma has a characteristic appearance which is not likely to be confused with that of any other neoplastic tissue. The color is dull white. The tissue is soft and tends to be somewhat friable. In some instances the vascular bed of the tumor masses may be congested, contributing a distinct pink cast to the color. An irregular infiltrative growth is typical of myelocytoma, and while localized masses may be found near bones, the growth in organs, as the liver, spleen, kidney, and lung, is usually diffuse. The liver, spleen, and kidneys, when affected with myelocytoma, become enlarged. However, the hypertrophy of these organs is not as marked as is commonly true in lymphocytoma.

The lesions of myelocytoma consist of infiltration with monotonously unvarying myelocytes. These cells, as previously mentioned, are similar to normal myelocytes found in the bone marrow and ectopic foci of myelopoiesis. Their nuclei are large, vesicular, and usually eccentric in position and tend to be round or oval in outline, although often their shape is distorted by compression. A distinct nucleolus is commonly present. The cytoplasm is filled with acidophilic granules so tightly packed that their shape cannot often be distinguished, and although the granules are usually spherical, spindle-shaped granules may be noted in some cells. Imprint preparations made by touching a glass slide to the cut surface of fresh tumor material can be stained with a polychrome blood stain after drying in the air. Such a preparation may be compared with similar ones made of the blood or bone marrow. With such a stain the large nuclei have a very fine arrangement of the chromatin and parachromatin, and the cytoplasmic granules, while predominantly acidophilic, are occasionally basic in reaction. These basic-staining granules represent a pre-acidophilic stage and later become acidophilic.

The myeloid tissue of the bone marrow becomes converted into a mass of tissue indistinguishable from foci of the tumor situated elsewhere. From the structure, it must be considered as a neoplastic process in the marrow. The involvement of the bone marrow appears to be a constant feature and may occur in every case of myelocytoma. Mathews (1929b) and Olson and Bullis (1942) found the condition

to exist in every case examined. In this respect, marked similarity exists between myelocytoma and granuloblastic leukosis. What appears to be a fundamental difference is that the neoplastic cells of myelocytoma are of a relatively later stage of development than those of granuloblastic leukosis. A careful comparative study of the minute structure of the bone marrow in myelocytoma and in leukosis will reveal this difference.

Fairly frequently, abnormal myelocytes gain access to the blood stream. The morphologic characteristics of these cells are similar to those of the cells in the extravascular foci of tumor. A distinct increase of the number of heterophils in the blood may be noted also. Probably the extent of involvement of the blood will vary during the course of the disease, although this aspect has not been studied carefully.

Metastasis and spread. Although Mathews expressed the belief that myelocytoma is a primary tumor of the myeloid elements in the bone marrow, there are other reasons, as mentioned previously, to suggest that myelocytoma may develop simultaneously in several widely scattered regions. The possibilities for such development have been mentioned under the heading of histogenesis. When the process is once initiated, further development is infiltrative. Nerves, muscle, and bone may be invaded by the infiltrative growth of the tumor. Cartilage alone seems to be capable of resisting growth of the tumor, and this phenomenon may be studied readily when the trachea is involved. The presence of tumor cells in the general circulation provides a means of dissemination of the tumor; however, the importance of metastasis in the disease cannot be estimated.

Special feature. Mathews made unsuccessful attempts to transmit spontaneous myelocytoma. The Strain 2 agent developed by Furth (1933) has caused a neoplasticlike process, which he refers to as myelocytomatosis, in addition to lymphomatosis and endothelioma. The relation between this experimentally produced

disease and spontaneous myelocytoma is not known. Nyfeldt (1934) reported development of a strain of leukosis in which leukemic myeloblastosis (granuloblastic leukosis) was the predominant type, although a few cases of aleukemic myeloblastosis (myelocytoma?) were also found in experimentally inoculated chickens. The occasional finding of myelocytoma as an enzootic in a given flock suggests the possibility of a common factor or factors as responsible for such an outbreak. For the present very little information is available on this point.

Diagnostic characteristics. Only brief comment is necessary to re-emphasize the comparative ease of recognizing myelocytoma. The color of the tumor and the distribution of lesions are features so characteristic of the disease that most cases of myelocytoma can be identified on macroscopic examination (Table 32.5).

Fowl leukosis. The term "leukosis" has been used with a wide variety of interpretations in connection with avian diseases. It is used in this section in a restricted sense to indicate the single entity briefly characterized in the following paragraph. Other applications of the terms "leukosis" and "leukoses" are noted in Chapter 19.

Fowl leukosis is a disease of the myeloid tissues in which the precursors of erythrocytes and granulocytes are stimulated to unrestricted multiplication. The apparently functionless autonomous growth of myeloid tissues serves to characterize fowl leukosis as a neoplastic disease. The neoplastic character of the immature blood cells is also illustrated by their tendency to become immobilized within the vascular bed of certain organs such as the liver, spleen, and kidney. In these situations they display proliferative growth outside the confines of the bone marrow where under normal conditions they would ripen into mature blood cells before being released into the circulation. The tendency for immobilization has been called leukostasis.

A thorough review of the extensive literature on fowl leukosis is beyond the scope of this section. Such a review was

made in 1940 by Olson and brought up to date by Darcel (1957). The morbid anatomy of naturally acquired fowl leukosis is not different from that produced experimentally. In fact, much of our knowledge of this hemoblastic neoplasm has been gained from study of the experimentally produced forms of the disease.

Histogenesis. A consideration of the histogenesis of fowl leukosis must be based largely on evidence obtained from various experiments dealing with the transmissible forms of the disease. The stimulus responsible for the development of this condition is an ultramicroscopic agent present in the tissues of affected birds. Such an agent was demonstrated first by Ellermann and Bang in 1908. The agent of fowl leukosis can be demonstrated in a spontaneous case of the disease only by reproduction of fowl leukosis in other birds following the experimental introduction of the causative agent.

Although much work has been done with fowl leukosis, the site of inception and the mode of action of the causative agent remain unsettled. A review of the literature has revealed many facts and opinions with regard to the pathogenesis of this malady. Not all strains of the agent are similar. Some appear restricted in action and cause only one form of disease (such as erythroblastic leukosis); some may cause both erythroblastic and granuloblastic leukosis; and some may cause leukosis and fibrosarcoma. The production of either erythroblastic or granuloblastic leukosis by a single agent is not difficult to harmonize with the hypothesis that the agent may attack a stem cell (hemocytoblast) common to both cell lineages. The form of disease which develops apparently depends on either the reactivity potential of the affected stem cell or the ability of the agent to influence the line of differentiation of the stem cell. Extensive studies on leukosis agents producing myeloblastosis and erythroblastosis have led Beard and his co-workers (1956, 1957) to conclude that these agents are distinct though closely related biological entities.

The complexity of the situation is illustrated by a report of Atanasiu (1952) working with the presumed pure strain SK of Engelbreth-Holm in chicken embryos. Most of 556 intravenously inoculated embryos developed erythroblastic leukosis. In addition there were 6 cases of myeloblastic leukosis which on subsequent passage reverted to the erythroblastic form.

The problem introduced by those agents of leukosis capable of also producing fibrosarcoma is more difficult to understand. The hemocytoblast and the fibroblast are related; yet this relation is somewhat distant, and it is rather difficult to believe that fibrosarcomas develop from hemocytoblasts stimulated by the agent of leukosis. The histogenesis of tumors produced by other agents such as the agent of the Rous sarcoma is not settled conclusively, although it is believed that the fixed or free histiocyte plays an important role in the process. Perhaps the agent responsible for leukosis may act in a similar way in the production of fibrosarcoma. Jármai (1935) explained the sarcoma-producing action of the agent of leukosis by suggesting that it had histotropic tendencies in addition to hemotropic tendencies, the latter being the more pronounced. Engelbreth-Holm and Rothe Meyer (1935) have advanced the conception that the different types of disease are caused by a selective action of the different agents of leukosis. For example, leukosis and fibrosarcoma are caused by an agent which attacks a mesenchymal cell capable of forming either blood cells or fibroblasts; those agents causing either erythroblastic or granuloblastic leukosis attack a cell common to both; and those agents causing only erythroblastic leukosis attack a cell already committed to that cell lineage. Carr (1956) has reported multiple nodules of renal adenocarcinoma in some chickens inoculated when less than 2 weeks of age with the ES-4 leukosis strain of Engelbreth-Holm. The size of the infective dose was also a critical factor.

Frequency. Fowl leukosis is usually a

sporadic disease among chickens and ordinarily affects birds more than 6 months of age. The average age of fowls suffering from leukosis studied by us has been approximately 1 year. Hamilton and Sawyer (1939) observed an unusual situation in which 53 of 231 chicks aged 30 and 39 days became affected with the disease within a period of 2 weeks. Olson and Bullis (1912) found 17 cases among 2,304 chickens more than 6 weeks of age, an incidence of 0.74 per cent.

Some evidence seems to suggest that in certain breeds (for example, Barred Plymouth Rock) of chickens, fowl leukosis is more likely to develop than in other breeds. Whether this is due to inherent characteristics of certain families within a breed or is a characteristic of all families of the breed is not known. Most if not all breeds are susceptible to transmissible strains of the agent of leukosis, and the spontaneous disease has been found in many different breeds.

There appears to be some relationship between the season of the year and the occurrence of the disease. Such relationship often has been suggested in the literature and is worthy of study. However, a number of factors serve to complicate such a study. In Denmark the disease appeared most often in the first quarter of the year; in Germany during the autumn, winter, and spring; in Japan in the late spring; and in Hungary in the autumn and winter months. In Massachusetts it appeared most often in birds examined during the second quarter of the year. Engelbreth-Holm and Rothe Meyer (1932) noted a seasonal effect on the results following inoculation of chickens with the agent of leukosis. A more severe form of the disease was noted in the summer months, and the disease developed in a higher percentage of adult birds during April and May than during October and November. Jármai (1938) observed a longer interval between inoculation and death of experimentally inoculated birds in the first half of the year than in the last half.

Gross and microscopic description. Fowl leukosis is fundamentally a disease involving the myeloid tissue of the bone marrow. Pathologic changes in other organs or tissues are secondary to the basic process in the bone marrow. With this simple conception in mind, the varied aspects of fowl leukosis as noted in other parts of the body may be understood readily.

The disease begins as a neoplastic proliferation of unripe erythrocytes or granulocytes. At first the process may resemble marked hyperplasia of myeloid tissue, but soon the normal boundary lines between intravascular erythropoiesis and extravascular granulopoiesis are so disturbed that they can no longer be distinguished. The neoplastic blood cells gain access to the circulation and are released from the diseased marrow in ever-increasing numbers. The tendency for these cells to become lodged in the capillary bed of certain organs has been mentioned previously. In such regions of leukostasis, the neoplastic cells continue to multiply and may rupture the vessel wall and infiltrate the parenchyma of the organ or tissue. Sometimes the lumen of the vessel may be filled with leukotic cells to such an extent that they constitute a thrombus and lead to infarction of the region supplied by the blood vessel.

In fowl leukosis the bone marrow is grayish-red and fills the marrow cavity. Johnson (1934) has called attention to the fact that in a normal bird the bone marrow space of the humerus contains fat and air spaces, whereas in most cases of leukosis the fat and air spaces are replaced by active myeloid tissue. A lining membrane of osteoid tissue may develop immediately inside the dense shaft of the long bones in cases with a protracted course. The intense hyperactivity of myeloid tissue can be studied best in histologic sections prepared from marrow of the long bones. The shaft may be split on its longitudinal axis to allow direct action of the fixative on the marrow. After fixation is complete, a segment of marrow may be embedded in the usual manner. The mar-

FIG. 32.10 — Erythroleukosis showing marked engorgement of the capillaries of the liver by immature erythroblasts. ×195.

row sinusoids are distended with unripe cells, and, likewise, the intersinusoidal tissue consists of unripe granulocytes. The relative amount of each determines the type of leukosis. That is, in the erythroblastic form, erythropoiesis is more marked; and in the granuloblastic form, the intersinusoidal tissue is the more severely affected.

The liver, spleen, and kidneys are the visceral organs of predilection in which the leukotic blood cells tend to lodge and proliferate (Fig. 32.10). The proliferation leads to generalized enlargement of the affected organ. In general this enlargement is not as marked as usually is seen in diffuse lymphocytoma. Microscopically the leukotic cells are confined largely to the vascular bed. The parenchyma of the organ may be reduced by compression from distention of the vascular bed with masses of neoplastic cells. The color of the involved visceral organs is usually pale because of the anemia. Sometimes small white foci may be present and represent localized accumulations of leukotic cells.

Fowl leukosis is associated with a tendency to hemorrhage, probably the result of an early and marked reduction of number of circulating thrombocytes. Hemor-

rhages may be noted in the loose areolar tissues and in the mucosa of the intestine.

The circulating blood is affected in nearly all cases of fowl leukosis. In experimental leukosis, cases may be observed in which the process is well developed in the bone marrow, and death occurs before the leukotic cells gain access to the circulation. Such cases are called incipient leukosis and represent a rapid acute form of the disease. All forms of leukosis may be found in the blood in varying numbers (Fig. 32.11). Usually the first change to be observed is a decrease of the number of thrombocytes. A decrease of the number of erythrocytes and of the amount of hemoglobin is followed closely or sometimes preceded by the appearance of immature cells. The blood picture is subjected to marked variations during the course of fowl leukosis. The microscopic picture of granuloblastic leukosis may sometimes change to that of erythroblastic leukosis. In some instances the blood picture may become apparently normal and remain so, suggesting recovery. Actual recovery of a spontaneous case has not been observed, although such a possibility may exist. In other cases periods of remission may be followed by

FIG. 32.11 — Blood films of chickens affected with leukosis showing marked differences between the myeloid and the erythroblastic form of the disease. (left) Myeloid leukosis. (Right) Erythroleukosis.
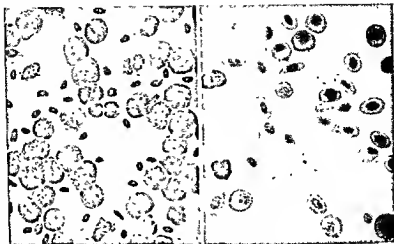
the reappearance of pathologic cells in the blood, and the disease eventually proves fatal. Although the foregoing impressions were obtained from experimental data, comparable changes occur in the naturally acquired disease.

Many bizarre and unusual forms of blood cells may be seen in leukosis. Furth (1931) and Oberling and Guérin (1934a) have published excellent colored plates illustrating the various types of blood cells seen in the blood of chickens suffering from fowl leukosis.

Special features. The relative ease with which fowl leukosis may be transmitted by means of the agent of leukosis suggests that spontaneous cases may develop as a result of natural exposure to the causative agent. Wickware (1946) found no evidence of transfer of leukosis to chicks hatched from pullets that had recovered from experimentally produced leukosis. Various experiments had indicated that not only fowl leukosis, but also the transmissible connective tissue tumors of chickens, are not contagious. However, Burmester, Fontes, and Walter (1960) found that contact transmission of Rous sarcoma could occur. Various ectoparasites have been shown capable of obtaining the agent from diseased birds and retaining it in an active form, but spread of the disease by such means can explain the development of few if any cases of leukosis. The spontaneous,

endogenous origin within the host has been suggested for the agents of leukosis and transmissible connective tissue tumors. In this respect these agents would be entirely different from the filterable viruses of contagious diseases such as fowl pox and laryngotracheitis. The concept of endogenous origin receives support in the different behavior of the many strains of the agent of leukosis, suggesting a lack of similarity. Tumors have been induced in chickens by carcinogenic chemicals, and if a tumor-producing agent separable from living cells could be demonstrated in these chemically induced growths, the evidence would support the hypothesis of endogenous origin of such agents. Only contradictory evidence is now available, and the latest report tends to deny the existence of such an agent in artificially induced tumors (Murphy and Sturm, 1941a).

Fowl leukosis is a disease peculiar to chickens. Only one spontaneous case of the disease has been reported in another species of fowl. This case occurred in a small parakeet (*Melopsittacus undulans*) and was described by Jármai (1939). The specificity of action of the agent of leukosis is probably only relative since the disease has been produced experimentally in pheasants, turkeys, and guinea fowl.

Natural resistance to the agent of fowl leukosis may be found in some chickens experimentally inoculated, and those

which recover from the experimental form of the disease also have a relative degree of immunity.

Diagnostic characteristics. The typical case of fowl leukosis is characterized by pale, watery blood which clots slowly, moderate enlargement of the liver and kidneys, marked enlargement of the spleen, and petechial hemorrhages in the loose areolar tissue and in the intestinal mucosa. The myeloid tissue fills the bone marrow space, replacing all fat cells and is grayred to dark red. Examination of smears of the blood will reveal abnormal numbers of immature cells.

In the diagnosis of leukosis, care must be exercised to differentiate it from other entities such as secondary anemias, granulomatous processes, and other neoplastic diseases. Histologic examination of the myeloid tissue should be regarded as the basic requirement for the diagnosis of fowl leukosis, and in some cases this procedure must be supplemented by a microscopic study of other organs as well (Table 32.3).

MELANOMA

Melanoma is a pigmented tumor whose black color is due to the presence of melanin granules in the cytoplasm of the cells. Histologically, the melanin appears as fine, dustlike, yellow-brown particles which may become so concentrated as to obliterate entirely the structure of the cell. The pigment is produced by melanoblasts which produce an enzyme capable of transforming the colorless precursor of melanin into pigment. By means of the "dopa" (dihydroxyphenylalanine) reaction this enzyme can be detected and thus the melanoblasts identified. Melanin granules may be engulfed by phagocytes which become simply carriers (melanophores) of the pigment. The histogenesis of melanoblasts is unsettled, and, according to the present conception, they may have either a mesodermal or a neural derivation (Boyd, 1938).

Excessive pigmentation with melanin without neoplasia may occur and is known as melanosis. Goldberg (1919) described

a case of generalized melanosis in a turkey and cited a similar case observed by Lewin in a chicken. According to Kukleuski (cited by Reinhardt, 1930), pigmentation of the gonads, oviduct, thymus, thyroid, skin, and marrow is often marked in Japanese and Siamese Silky chickens, which normally have a pigmented skin. Reinhardt (1930) commented that pigmentation of one or both testes is fairly common in "singing" birds. Melanosis of the peritoneum occasionally may be noted in the chicken.

Few pigmented tumors of chickens have been described. Reitsma (Hoogland, 1929) and Goldberg (1919) each reported a melanoma, probably primary, in the ovary of a hen, which spread to the serosa of the abdominal viscera. In the case described by Goldberg, the tumor resembled a cavernous angiosarcoma except for the pigmentation. McGowan (1928) described three cases of melanoma in the chicken. Two of the pigmented tumors occurred in Black Leghorn chickens and the other in a Rhode Island Red bird. In all of these cases the tumor was believed to have originated in the ovary. Only one appeared epithelial. The other two were described as similar to the Rous sarcoma. In McGowan's cases numerous implants of pigmented tumors were found on the serosal surfaces of the visceral organs. Olson and Bullis (1942) observed a small pigmented tumor at the base of the tongue which was diagnosed as melanoma. Ball (1945) reported a melanoma of the iris in a 2-year-old hen that died with lymphomatosis.

TUMORS OF NERVE TISSUE

If lymphocytoma is excluded, neoplasia of nerve tissue of the chicken would appear from the literature to be relatively infrequent. Jungherr and Wolf (1939) reviewed the literature on primary neural neoplasms of animals and found only three cases in the common fowl in which the diagnosis of glioma could be accepted. They described two additional cases. All were regarded as astrocytomas. Multiple gliomas have been found in birds with

FIG. 32.12 — Glioblastoma multiforme illustrating irregular cell outlines and glia fibrils. ×480. (Courtesy of Cecil Jackson, Ghana Academy of Sciences, Achimota, Ghana, and of the Director of the Onderstepoort Veterinary Research Institute, South Africa.)
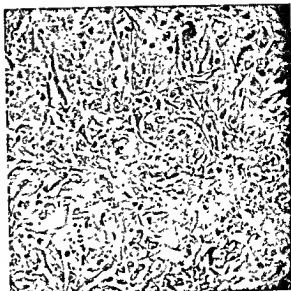
FIG. 32.13 — Showing multiplicity of focal areas of avian gliomatosis. From foci of encephalitis, such as occur in the upper and central left areas, there arise by transition definite gliomatosis neoplasia. An example is the large oval area at the extreme right. ×100. Giemsa stain. (Courtesy of Cecil Jackson, Ghana Academy of Sciences, Achimota, Ghana, and of the Director of the Onderstepoort Veterinary Research Institute, South Africa.)

toxoplasma infection (Erichsen and Harboe, 1953). Jackson (1954) has presented a series of 120 intracranial lesions from chickens with 109 gliomas (Fig. 32.12). He believes that gliomas are more like glioblastoma (spongioblastoma) multiforme rather than astrocytoma and that they represent foci of encephalitis in which glial proliferation becomes exaggerated and assumes a neoplastic character (Fig. 32.13). Jungherr and Wolf also discussed neoplasms reported from other birds and regarded a glioma found in a parakeet and a ganglioneuroma described in a sparrow as of neural origin. They stated that the apparently low rate of incidence of neural tumors in animals is perhaps due to the infrequent complete examination of the central nervous system at necropsy.

Jackson (1936a) has described multiple neurofibromatosis in the chicken, and Olson and Bullis reported five cases of neurogenic sarcoma. These tumors are mentioned in the section dealing with connective tissue tumors. Cole in 1946 reported a case of retinoblastoma.

No attempt will be made to discuss the classification or characteristic features of tumors of the nervous system. Those seeking such information are referred to the chapters on neoplasms in Lichtenstein (1949).

In some instances the brain or spinal cord may be affected by metastatic growth of neoplasms situated elsewhere. The involvement of nerves with lesions of lymphocytoma is fairly frequent in that disease. The significance of these lesions and their association with fowl paralysis are discussed in the section on lymphocytoma.

TUMORS OF EPITHELIAL TISSUES

Papilloma. A papilloma is a benign epithelial tumor composed of fibrous cores or projections which are covered by layers of epithelial cells. These tumors are frequently multiple, and the brushlike or cauliflowerlike structures are often spiny to the touch.

Microscopically, papilloma is a simple structure usually consisting of a few to many separate units or projections, each with a fibrous core that is covered to a variable depth by compactly arranged epithelial cells. The cells nearest the stroma are the least mature, and between the various papillae and on the surface there is frequently present a horny deposit known as keratin. Keratin is the product of the more mature squamous epithelial cells. Characteristically, these tumors grow in an outward rather than an inward direction.

The tumors are sometimes seen on the comb, feet, and wattles of fowls, but they occur much less frequently in fowls than in certain mammals. Olson and Bullis (1942) observed a case of multiple papillomatosis of the esophagus of a chicken. The lesions appeared as small, grayish nodules, some of which were hemorrhagic, in the mucosa. We observed one instance in a pigeon in which there occurred diffuse warty growths in the skin adjacent to the beak and around the eyes. The literature contains but slight mention of papillomas in chickens, and one must conclude that their occurrence is extremely infrequent.

Papilloma of the skin and oral cavity in mammals frequently is contagious and easily transmitted owing to a causative factor that in some instances has been definitely established to be a virus. So far as we know, papillomas of chickens due to agents of a viruslike nature have not been reported.

Adenoma. Adenoma may be defined as a benign epithelial neoplasm in which the structural pattern resembles that of a gland. Any tissue containing glandlike structures normally or aberrantly may give rise to an adenoma. These tumors usually occur singly. Rarely, multiple adenomas may appear.

Adenomas are among the less frequent tumors of chickens, being much less common than the malignant epithelial tumors. Eber and Malke (1932) reported the occurrence of 16 adenomas among 253 tumors of fowls. Of those in chickens, the sites of occurrence were as follows: liver,

6 cases; proventriculus, 1 case; gizzard, 1 case; intestines, 2 cases; ovary, 1 case; and oviduct, 1 case. According to Heim (1931), Joest and Ernesti described a cystic form of adenoma (cystadenoma) in the region of the crop. Although the exact situation of origin was not determined definitely, origin from the thyroid was considered. A few cases of adenoma of the ovary have also been described (Heim). Olson and Bullis observed 1 case of fetal adenoma of the thyroid and 1 case of adenoma of the feather matrix in chickens. The same authors recorded a papillary cystadenoma of the mucosa of the posterior portion of the gizzard in a chicken.

Unless adenoma occurs in a situation where its presence or size may provide a mechanical interference with the proper functioning of contiguous tissues, this variety of neoplasm in chickens is unlikely to have any appreciable effect on the host. Malke, according to Heim (1931), recorded an obstructing adenoma of the cecum. Babic (1931) described multiple adenomatous polyposis of the intestine in a chicken. Such a condition could lead to disturbances of elimination. Should adenoma arise in certain endocrine structures, abnormal physiologic effects may ensue.

Being benign, adenoma never infiltrates the surrounding tissues and does not metastasize. Should an alleged adenoma exhibit these features and especially should metastasis occur, the tumor can no longer be considered benign but should be recognized as malignant. The malignant form of adenoma is designated "adenocarcinoma."

In appearance adenomas may be expected to be encapsulated, nodular, and firm to soft. Opportunity for adenoma to become cystic is provided by the glandlike nature of the parenchyma. Since a duct system for the natural egress of secretory substances is missing, the products of the cells frequently accumulate and produce small to large cysts. Such tumors are often called "cystadenomas."

Microscopically, an adenoma presents the appearance of a gland, a duct, or a tubular structure (Fig. 32.14). Alveolar spaces may be present, or the parenchymal cells may appear as compact masses. In nearly every instance the structure of the tumor bears a resemblance to the normal tissue produced by the parent epithelial cells from which the parenchymal cells of the tumor were derived. The stroma consists of fibrous connective tissue in which are found blood vessels. The stroma may occur in a promiscuous, nondescript fashion, or it may be disposed as septa or ill-defined trabeculae which serve to separate the tumor into irregular lobules.

Adenomas may be identified readily if one keeps in mind certain salient features: 1. Adenomas originate in situations where glandlike structures occur normally. 2. They usually occur as single tumors. 3. They do not infiltrate the adjacent tissues or set up distant metastatic growths. 4. When properly removed they do not recur.

Carcinoma. A carcinoma is a malignant neoplasm composed of epithelial cells and a stroma of connective tissue. The latter provides a supportive structure for the epithelial cells and for the vascular channels inherent to the part. The cells of the carcinoma proliferate in an atypical and lawless manner, have a tendency to infiltrate and destroy the contiguous tissues, and may and often do set up secondary or metastatic foci. Although all malignant tumors of which the type cell is epithelial in origin are properly referred to as carcinomas, certain characteristic structural differences occur that make it desirable to separate carcinoma into several distinct morphologic types. These include adenocarcinoma, in which the parenchymatous cells assume a glandular or ductlike arrangement; squamous cell carcinoma, composed of diffuse masses or compact collections of cells that arise from the epidermis or the mucosa of the esophagus, the mouth, or the pharynx; papillary carcinoma, which has a rough cauliflowerlike surface with the tumor cells arranged in fingerlike sheets; and hepatic cell carcinoma, which arises from the cells of the

FIG. 32.14 — Adenoma arising from the bile ducts of the liver. ×120.

parenchyma of the liver which have undergone autonomous transformation. Tumors of chickens analogous to the so-called basal cell carcinoma of human beings presumably occur infrequently. Olson and Bullis (1942) encountered one case in their material. This is described in the subsequent text. Other special types of carcinoma may arise from the thyroid, the adrenal, the ovary, the kidney, and the pancreas. These constitute only a partial list of the tissues that may give rise to carcinoma. The wide distribution of epithelial tissues throughout the body provides numerous potential situations for the origin of carcinomas.

Frequency. Among 199 neoplasms of chickens mentioned by Hoogland (1929), 33, or 16.6 per cent, were carcinomas. The predominance of sarcoma over carcinoma was illustrated in Hoogland's series, there being 93 tumors of sarcomatous character. Hoogland's list of chicken tumors did not include those of so-called leukotic character (lymphocytoma, myelocytoma, and leukosis). Eber and Malke (1932) observed 29 carcinomas among a total of 239 tumors of chickens. This represents an incidence of approximately 12 per cent. As was true in Hoogland's series, Eber

and Malke probably did not include leukosis in listing the respective neoplastic diseases in their material. However, it is likely that many, if not all, of the so-called round-cell sarcomas mentioned by Eber and Malke were in reality lymphocytomas. As is true with most other reports on neoplasia of chickens, Eber and Malke's material contained many more "sarcomas" than carcinomas. Of the 259 tumors of chickens examined histologically, 167, or approximately 70 per cent, were designated as sarcoma.

Babic (1931) described 16 cases of carcinoma of the chicken, in 10 of which the tumor was primary in the ovary. In 3 the tumor was primary in the skin, in 2 the kidney was the site of origin, and in 1 instance the growth arose in the testes. The tumors occurred in a group of 61 neoplasms collected from several different species of birds.

Goss (1940b), who reported on the types of neoplasia among 7,408 chickens examined at necropsy, found tumors in 1,445. Among 1,104 examined microscopically there were 991 designated "leukotic tumors" and 77 carcinomas. In relation to the total number of tumors listed (1,104), carcinomas represented 7.0

per cent; if the "leukotic tumors" are excluded from the total number, carcinomas represent approximately 68 per cent of the neoplasms examined. Goss's data are of especial interest because of the fact that 70, or approximately 91 per cent, of the total number of carcinomas found were ovarian in origin.

Olson and Bullis (1912) made a study of avian neoplastic material that occurred in 365 chickens. A total of 384 tumors, including those of hemoblastic origin, were found, and 24, or approximately 6.2 per cent, were epithelioblastomas.

From the figures presented, it is evident that dependable information concerning the predictable rate of occurrence of carcinoma in chickens is meager.

Relation to age. There do not exist adequate data to enable one to state definitely the relation of the incidence of carcinoma to the age of the affected bird. Indications are that the majority of carcinomas of chickens occur in adult rather than in young birds. Most birds that have carcinoma are 1 year or more of age. It should be kept in mind, however, that the age at which the neoplastic process began was much earlier. More than 4 years were required for development of epithelial tumors in liver, ovary, and oviduct of chickens which received a carcinogen (2 acetamidofluorene) daily for 3 months beginning when the birds were 6 months old (Campbell, 1955). Methylcholanthrene applied to the wall of the crop produced carcinomas in chickens in 3 to 5 years (Peacock and Peacock, 1956) but, when applied to the skin, epithelial proliferation developed in 2 to 3 months and these regressed (Rigdon and Brashear, 1954).

Sites of occurrence. A fairly complete résumé of the literature on occurrence of carcinoma in chickens is to be found in the treatise by Reis and Nóbrega (1955a). As mentioned previously, carcinomas may arise wherever epithelial tissues occur. Although the epidermis and the mucous membranes constitute the greatest amount of epithelial tissue in the body, in the chicken these tissues do not give rise to the largest number of carcinomas. In chickens most tumors of this character arise from the ovary.

From information obtained from the literature and from data supplied by our own collection, the occurrence of carcinoma in the various anatomic situations in chickens will be described briefly.

1. *Integument.* By far the greatest number of carcinomas of the integument of chickens that have been reported have affected the foot and shank or more specifically the skin overlying the metatarsus. Other situations in which carcinoma of the skin has been reported include the anal region, breast, and the region overlying the femorotibial articulation.

One instance of a tumor that had many of the characteristics of a basal cell carcinoma was reported by Olson and Bullis (1912). The tumor was situated in the skin immediately over the left eye. It was a nodular mass 1 cm. in diameter by 6 mm. thick. The tumor was first observed 1 month before the chicken was killed for necropsy. Microscopically, the mass consisted of several indistinctly lobulated, compactly disposed epithelial cells with moderately basophilic cytoplasm. The tumor was situated largely in the corium but had broken through the epidermis at one point. The structure was richly vascular, and some hemorrhage had occurred. Mitosis was not observed. Metastasis had not occurred. The tumor was diagnosed as carcinoma of the feather matrix. For a review of the different reports of carcinoma of the skin of chickens up to 1930, the paper by Heim (1931) may be consulted. Other cases are mentioned by Pohl (1926), by Babie (1931), and by Jackson (1936a, page 434). An extensive review of the literature pertaining to tumors of the skin of birds will be found in the paper by Abels (1929).

In view of the fact that these tumors of the integument have all the morphologic characteristics of a malignant growth, it is somewhat surprising that they apparently seldom if ever metastasize (Fig. 32.15).
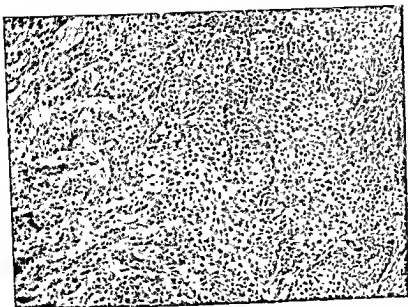
FIG. 32.15—Squamous carcinoma of the skin of the neck of a chicken. Metastasis was not demonstrated, ×120.

Duran-Reynals (1946b) was unable to transplant a localized skin gland adenoma of the wing to other chickens. In the cases we have observed, the neoplasms have remained localized, and in the cases reported previously by others, metastasis has rarely been demonstrated. Structurally, these tumors would appear capable of setting up metastatic foci at a distance from the original lesion. Yet in the many cases reviewed by Abels (1929), metastasis was recorded in only three cases of carcinoma of the skin of chickens.

2. *Alimentary canal.* A few instances have been recorded of the occurrence of carcinoma within the oral cavity of chickens (Heim, 1931, listed several cases). One occurred in our collection. The tumor was a carcinoma of the epidermoid type and occurred in the pharynx of a 2-year-old hen. Although the tumor was locally infiltrative and destructive, metastasis could not be demonstrated.

The literature, according to Heim (1931), yields three cases of squamous cell carcinoma of the esophagus.

Carcinoma of the proventriculus and gizzard appears to be an extremely rare manifestation of the disease. Our material did not contain any specimens from these organs, and the cases reported in the literature are few. Babic (1931) described a medullary carcinoma of the proventriculus. Zannini (Heim, 1931) is said to have observed an adenocarcinoma of both the proventriculus and the gizzard. "Cylindrical cell" carcinomas of the gizzard that had not metastasized were reported by Schöppler (1913) and by Prospero (Heim, 1931). The report of Prospero was not available for review, so that we are unable to comment concerning this case. In Schöppler's case the tumor was considered to have arisen from the glandular elements of the pyloric portion of the gizzard.

3. *Intestine.* The tendency of many primary malignant tumors of the abdomen to spread by direct continuity or by serosal implantation to all of the serous surfaces of the abdomen frequently makes it difficult to ascertain with certainty from what site a given tumor may have arisen. The occurrence of serosal implantations is especially characteristic of ovarian carcinoma, and the resultant widespread distribution of the tumorous tissue may obscure entirely the primary site of origin or lead to false conclusions regarding the primary situation of the tumor. Unless one can demonstrate the primary lesion

in the intestinal mucosa, it would be unwise to claim that a carcinoma of the intestine is present. When the serous covering or even the muscle wall of the intestines is involved with an epithelial glandular type of malignant lesion, discretion should be exercised in concluding that the site of origin was the intestinal mucosa. As a matter of fact the likelihood that such tumors originate in the ovary is much greater than that of their origin in the intestine.

Adenocarcinoma of the duodenum has been reported by Petit and Germain (Pohl, 1926) and by Ehrenreich and Michaelis (1906), and for the "intestine" by Hoogland (1929) and by Jackson (1936a, p. 160). In Jackson's case, metastasis to the liver had occurred. An unusual case was that of Joest and Ernesti (Heim, 1931), in which a medullary carcinoma involving the ileum and ceca was associated with another primary carcinoma of the cloaca. One of the specimens in our collection was of some interest. The tumor, which proved to be an adenocarcinoma, occurred in the mucosa of the ileocecal junction of a 2-year old White Leghorn hen (Fig. 32.16). The tumor was an elongated, roll-like structure about 0.6 cm. in height, and it involved about half of the circumference of the lumen. The tumor had produced some obstruction, but metastasis had not occurred.

According to Heim (1931) two cases of carcinoma of the colon or rectum of chickens have been described. In both instances metastasis had occurred to the liver.

The evidence indicates (1) that malignant epithelial tumors of the intestines of chickens are among the rarer avian neoplasms; (2) that carcinoma of the intestines may metastasize to the liver and lungs; (3) that in female chickens the occurrence of multiple neoplastic foci on the serosa of the abdomen should suggest a malignant lesion of ovarian origin rather than one from the intestinal tract; and (4) that to diagnose with certainty a carcinoma of the intestine, one should

FIG. 32.16—Primary adenocarcinoma of the ileocecal junction of a 2-year-old White Leghorn hen. The advancing neoplastic cells have penetrated the muscolaris mucosae. Distant metastasis was not demonstrated. ×110.

demonstrate the primary lesion in the mucosa of the gut.

4. *Accessory organs of digestion.* Among the accessory organs of digestion that have given rise occasionally to carcinoma are the liver and the pancreas.

Heim listed a few reports of epithelial tumors of the liver, but how many of the alleged cases were actually primary carcinomas of the liver is uncertain. Teutschlaender (Heim, 1931) reported two cases of carcinoma of the bile ducts, and Savage (1926) reported the occurrence of an adenocarcinoma of the gallbladder in a 3½-year-old Rhode Island Red hen.

Primary carcinoma of the liver may arise from two types of cells, the parenchymal liver cells and the epithelium of the bile ducts (Fig. 32.17).

Goss reported one case of carcinoma of the liver cells in a bird more than three
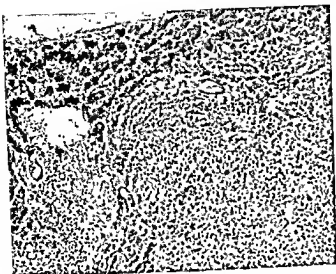
FIG. 32.17 — Hepatocellular carcinoma (hepatoma). The neoplastic liver cells are compressing the normal liver tissue by their expansive growth. In some cases, the neoplastic cells may occur in more orderly rows. ×150.

years old and also an adenoma of liver cells in another chicken. He also recorded 4 carcinomas of bile ducts. Olson and Bullis (1942) described 9 cases of benign hepatoma and 4 of cholangioma. In 3 instances the tumors of the bile duct cells were single isolated masses, and in the fourth case there were multiple tumors scattered throughout the liver, suggesting the malignant character of the neoplasm. An adenocarcinoma primary in the liver of a turkey was described by Babic (1931).

Campbell (1949) studied 22 cases of spontaneous carcinoma of the liver in ducks mostly from 4 flocks. Carcinoma of liver cells was most common (17 cases), while 1 case of bile duct carcinoma and 4 cases of mixed liver carcinoma were encountered. Graft transplants to the livers of other ducks were successful, but attempts to demonstrate a cell-free agent in filtrates or by egg embryo culture were negative. Liver injury with regeneration and metaplasia of bile ducts has been demonstrated in ducks fed a toxic peanut meal or aflatoxin for up to 4 weeks (Newberne, 1964). The same peanut meal or aflatoxin fed for 12 weeks to rats produced hepatomas and cholangiomas. A dietary carcinogen could be suspected in circumstances such as described above by Campbell (1949).

Olson and Bullis (1942) recorded having encountered 3 epithelial malignant lesions of the pancreas. Two of these were designated as carcinomas. Babic (1931) also listed as an adenocarcinoma. Babic (1931) also described an adenocarcinoma of the pancreas.

5. *Adrenal glands.* Mathews and Walkey (1930) described, in adult hens, 6 cases of pedunculated carcinoma which appeared to originate from the region of the adrenal glands. Although the ovary was involved in each instance, the histologic picture of the tumors was suggestive of the adrenal cortex. Mathews and Walkey, however, were not certain that the tumors had originated from this tissue. Metastasis was limited to the mesentery and to the visceral peritoneum and probably occurred as a result of spread by continuity.

Berner (1923) reported an extremely interesting case of hypernephroma or carcinoma of the right adrenal gland with secondary involvement of the serosa of the abdominal viscera and metastatic foci in the lungs. The bird was an 18-month-old female chicken in which the behavior and other characteristics of virilism had been

noted since the bird was 6 months of age. There had developed a male type of comb and spurs, and the gait when walking was particularly vigorous and malelike.

6. *Urogenital tract.* A case of medullary carcinoma of the kidney with metastasis to the liver and lung was found in a pheasant by Babic. He also reported a cystic adeno-carcinoma in the right kidney of a chicken. The latter case might be considered more properly as an embryonal nephroma. A medullary carcinoma of the testis of a chicken with metaplastic keratinization also has been described by Babic.

The ovary is undoubtedly the most frequent site of origin of carcinoma of the chicken. Carcinoma of the ovary of chickens has been described by many observers. Those especially interested may consult the following: Oshima and Roki (1925), Eber and Kriegbaum (1916), and Jackson (1936b). Heim reviewed the literature up to 1930. In Eber and Malke's series of 239 tumors of chickens, 29 were carcinomas. Fifteen, or approximately 52 per cent, of the carcinomas in this series were presumed to have originated in the ovary. Unlike many carcinomas of the ovary of human beings, which affect the ovary secondarily, most carcinomas of the ovary of chickens are primary in this organ. The disease in chickens usually has its inception in the functioning left ovary but may originate in the rudimentary right ovary.

Seifried (1925) described two cases of ovarian tumors in chickens which he designated "oophoroma folliculare." One tumor occurred in the rudimentary right ovary and the other in a left ovary which was otherwise normal. From a careful reading of Seifried's description of the two tumors, we believe that his Case 1 represents a granulosa tumor and not a Brenner tumor as he believed. Case 2 may also be a granulosa tumor, but the evidence is somewhat indefinite.

In the ovary, neoplasia may arise from (1) the germinal epithelium, (2) the Pflüger egg cords, and (3) the immature follicular epithelium. Fischel (1922) stated that most of the ovarian tumors in human beings are derived from primitive mesenchymal tissue which has divergent potentialities, and which in his opinion may give rise to sarcoma or to carcinoma. Babic expressed the belief that most ovarian carcinomas are derived from follicular epithelium. Carcinomas that arise in the oviduct are derived from the epithelium of the mucosa.

Notwithstanding the possibilities for origin of ovarian carcinoma just listed, it should be kept in mind that, with the possible exception to be mentioned, cytologic detail of carcinoma of the ovary does not bear any resemblance to any recognizable ovarian structure. A possible exception to this statement is to be found in the case of granulosa tumor of the ovary. Even with this neoplasm, however, there is little unanimity of opinion concerning its true genesis. Most carcinomas of the ovary are medullary in character.

Attempted transplantation of an adenocarcinoma of the ovary to other chickens by Duran-Reynals (1916b) was not successful.

7. *Other situations.* Information on the occurrence of primary carcinoma of the lung of chickens indicates that this organ is seldom affected. The only reference to primary carcinoma of the lung of the domestic chicken with which we are familiar is a report of a case by Apperly (1935). The right lung and most of the left lung were replaced by a fibrocaseous material, and there was present a small white nodule near the periphery of the liver. Microscopically, the appearance of the lesions in the lung and in the liver was essentially identical. The diagnosis was primary adenocarcinoma of the lung with metastasis to the liver.

Epithelial malignant lesions of the thyroid gland appear to be rare in the chicken. Elsner (Reis and Nóbrega, 1935a) described an adenocarcinoma, and Olson and Bullis (1942), a fetal adenoma in the thyroid of chickens.

Effects on the host. The effects on the welfare of an animal in which carcinoma develops depend on several factors. These

include (1) the inherent malignancy of the process, (2) the anatomic situation involved and the severity of the involvement, (3) the presence or absence of secondary foci, and (4) the duration of the disease.

Carcinomas of the ovary frequently spread by continuity or by implantation to the adjacent structures and especially to the serosa overlying the intestines. When this occurs, moderate to severe ascites eventually follows. As a matter of fact, the presence of ascites in an adult chicken should suggest the possibility of abdominal carcinomatosis or other malignant processes having a multiplicity of lesions.

It is of interest that although carcinoma of the abdominal cavity may be disseminated extensively throughout the viscera, frequently the condition appears not to interfere seriously with the general health of the bird except in the terminal stages. The disease has a protracted course, and in many instances is discovered in apparently healthy chickens at the time the bird is being "dressed" for food. Emaciation may be noted.

Hens that have carcinoma of the ovary eventually become nonproductive, although what degree of involvement of the ovary must occur before egg production ceases is not known.

Although the effect of most carcinomas, like that of many other malignant tumors, depends on the mechanical interference with normal function and on the destruction of normal tissues by the advancing neoplastic process, a few carcinomas occur that affect the host physiologically by producing an excess of hormonal substances. The masculinizing effect of arrhenoblastoma in the human being is well recognized, and if this or certain other specialized tumors occur in chickens, it would seem reasonable to expect an altered behavior on the part of the host. Friedgood and Uotila (1941) described several cases of virilism in chickens apparently due to hormones produced by tissue which they considered neoplastic. They diagnosed these cases as arrhenoblastomas. Virilism in a hen should lead to suspicion of such a condition.

Gross characteristics. The gross appearance of carcinomas varies greatly as to size, shape, and color. With few exceptions these tumors are attached diffusely to the surrounding tissues, and encapsulation, except in rare instances, is not discernible. Contact with the adjacent tissues is usually intimate, this feature being indicative of the characteristic invasive tendencies of these neoplasms. Those on the exterior, being subject to trauma, are readily infected, and as a consequence they may present hemorrhagic, necrotic regions. Their surface is usually irregularly roughened and may have a hard, tough, or cornified superficial covering due to the excessive amount of keratin derived from the squamous epithelial cells.

Carcinomas of the internal organs, especially those of the ovary or oviduct, are single or more frequently multiple, smooth, nodular, pinkish-gray or flesh-colored formations (Fig. 32.18). Multiplicity is frequently a striking feature of intra-abdominal carcinomas, with innumerable nodules of varying sizes and shapes occurring in subserosal situations, especially along the intestines and in the mesentery (Fig. 32.19). Ovarian and oviductal carcinomas may be soft or hard or, more precisely, cystic or solid. Cavities or cysts filled with a shiny mucinous or gelatinous substance are commonly a characteristic feature of these tumors.

Carcinomas of the mucous surfaces are often the site of extensive ulceration. The surface may appear raw and irregular, with evidence of recent bleeding. Signs of secondary infection are present. In situations where metastasis has occurred, as in the lung or liver, the tumorous tissue usually appears as firm, grayish-white foci. In the liver the metastatic foci may be limited to the serosa of the capsule or may occur firmly embedded in the parenchymal tissue.

Microscopic characteristics. Since carci-

FIG. 32.18 – Solid carcinoma of the ovary.
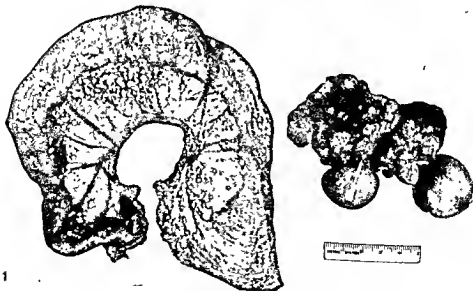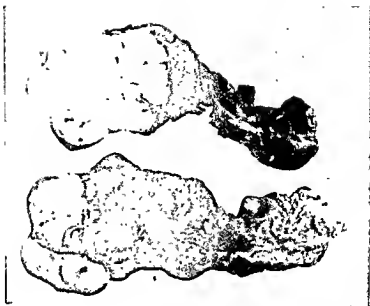
FIG. 32.19 – Adenocarcinoma of the ovary showing multiple implants on the serosa of the oviduct of the mesosalpinx.

nomas arise from many different types of epithelial tissue, the histologic pattern depends to some extent on the character of the parent epithelium from which they originate. However, most carcinomas have one important feature in common. This feature is the infiltrative and destructive character of their growth.

The various types of carcinoma that may be encountered can be described briefly as follows:

1. *Squamous cell carcinoma.* These tumors arise from the cells of the epidermis and consist of diffuse collections or compact masses of squamous epithelial cells. The epithelial cells are usually incompletely separated by a fibrous connective tissue stroma with nestlike groups or packets or fingerlike processes that extend into the surrounding nontumorous tissue. Many if not most of the epithelial cells show signs of immaturity, and mitotic division usually can be seen. Necrosis may be present, and lymphocytes and acidophilic granulocytes commonly infiltrate the tumor. The central portion of many of the compact groups of cells frequently presents a peculiar hyaline appearance due to the accumulation of keratin. Such a tumor may be referred to as a "pearl cell" or an epidermoid carcinoma.

2. *Papillary carcinoma.* Rarely a carcinoma grows away from, rather than into or toward, the body, presenting a rough, cauliflowerlike structure. Such a tumor consists of a central core of connective tissue from which are given off small papillae covered with several irregular layers of neoplastic epithelial cells.

3. *Adenocarcinoma.* This variety of carcinoma arises from tissues normally composed of or containing epithelial glandular structures. The tumor consists of fibrous stroma of variable dimensions which support one or more layers of cuboidal or columnar cells arranged in an alveolar or ductlike fashion (Fig. 32.20). Tortuous ductlike structures frequently are formed, as are cystic spaces containing gelatinous or mucoid material. The cells may assume a papillary type of growth, and, if the process is especially anaplastic, little tendency toward formation of alveoli or ducts may be evident. Instead, the growth appears as diffuse or solid sheets or nests of cells with slight resemblance to the parent structure from which the tumor arose.

4. *Other types of carcinoma.* Another variety of epithelial malignant lesion is that commonly referred to as hypernephroma of the kidney. This is a renal

FIG. 32.20 — Cystadenocarcinoma of the ovary of a chicken. Metastasis to the lungs had occurred. ×150.

carcinoma that arises either from the adult tubules or from foci of nephrogenic tissue that have persisted in the renal cortex. Hypernephroma of the kidney appears to be rare in chickens.

Olson encountered a hypernephroma in the kidney of a hen about 1 year old. The bird died with extensive lymphocytoma of the visceral organs. The hypernephroma was a well-encapsulated, firm, round mass in the posterior pole of the left kidney. The tumor was 4.5 cm in diameter. The cut surface was solid, and thin bands of connective tissue separated islands of yellow-tinged, gray-white tumor tissue which resembled the adrenal gland. The tumor was composed of large cells with a foamy cytoplasm which resembled those of the adrenal cortex. There was no evidence of virilism of the bird, and both adrenals were normal.

Metastasis. Carcinomas, being malignant neoplasms, have the tendency to infiltrate or invade the surrounding tissues, and if the circumstances are propitious, new foci of growth and destruction are likely to be set up in the immediate vicinity of the parent growth or in tissues distant from the primary lesion.

In carcinoma of mammals the usual route of metastasis is by way of the lymphatics, with the blood channels involved infrequently. In chickens the lymphatic route of metastasis is apparently little utilized. As a matter of fact, true metastasis of carcinoma in chickens as a consequence of the conveyance of tumor cells from one situation to another by way of vascular channels seldom occurs. Ovarian and oviducal carcinomas early become disseminated throughout the abdominal tissues, but this results as a consequence of implantation rather than by true vascular metastasis. Distant metastatic growths may be established from these tumors but are of exceptional occurrence (Fig. 32.21). A few instances have been observed in which carcinoma primary in the intestines has metastasized to the liver and to the lungs.

Diagnostic characteristics. Of primary importance in distinguishing carcinoma is the character of the tissue in which the tumor occurs. Being composed of epithelial elements, carcinoma should be thought of when neoplasia occurs in such tissues as the skin or the mucous membrane or in organs in which epithelial tissues are present. These tumors usually exhibit invasive or infiltrative tendencies

FIG. 32.21 — Metastatic cystadenocarcinoma of the lungs. The neoplastic process was primary in the ovary. Same case as Figure 32.20. ×60.

and frequently give rise by implantation, or less often by metastasis, to secondary foci. Microscopically, although the picture is subject to marked variation, carcinoma usually is characterized by the presence of immature epithelial cells growing in an atypical infiltrative manner without the apparent guiding influence that governs normal growth or repair.

MESOTHELIOMA

Neoplasia of the mesothelial cells covering the serous surfaces of the peritoneal cavity appears to be rare in the chicken. Olson and Bullis (1942) described one case of a 9-month-old pullet. In this case two pedunculated masses of tumor having a combined weight of 49 gm. were attached by stalks to the wall of the abdomen and the ovary. There was marked ascites. Eber and Malke (1932) mentioned four cases of what were probably mesotheliomas of the peritoneum. Eber and Malke designated these tumors as "endothelioma." In these cases there were numerous tumorous nodules spread over the serosa of the proventriculus, intestine, oviduct, and mesentery. In all cases microscopic examination revealed masses of neoplastic tissue composed of large cells which had a distinct fibroblastic character. These cells tended to form cylindrical strands, although there were regions in which the cells were loosely arranged.

Mesothelioma may arise from the serosa of the peritoneum or the pericardium. The structure of such tumors may be variable since the mesothelium is of mesodermal origin, and immature mesothelial cells may have the ability to develop into connective tissue cells. In the case of mesothelioma described by Olson and Bullis, the polyhedral cells tended to form sheetlike masses, although in some regions they appeared to be compressed to a spindle shape. The cases described by Eber and Malke as endothelioma might be considered as mesotheliomas in which the elements displayed more of the fibroblastic character than the tendency to form sheetlike masses.

Although mesothelioma appears to be a rare tumor in the chicken, it is important since it might readily become confused grossly with lymphocytoma or carcinoma. Careful study may be required to enable one to distinguish between these conditions.

MIXED TUMORS

For convenience we have classified certain tumors of varying degrees of complexity as mixed tumors. The less complex are those whose cells, although derived from more than one germinal layer, are in a rather advanced state of development and whose potential differentiation is subject to definite restrictions. The tumors of chickens in this category include thymoma and the so-called carcinosarcoma. A more complex group of mixed tumors is that known collectively as teratomas. These are also composed of cells derived from more than one germinal layer but differ from the less complex mixed varieties just mentioned in that the cells giving rise to a teratomatous growth have multipotent potentialities and are capable of unrestricted differentiation. In teratomatous tumors, therefore, one may recognize tissues representing all three germinal layers, some of which may have attained the specialized differentiation characteristic of adult tissues or organs. In chickens the most frequently encountered teratomatous tumors are those of the ovary, the kidney, and the testicle. A third group of complex tumors are those designated embryonal nephroma, which arise from primitive multipotent mesodermal cells that have divergent capacities for differentiation.

Thymoma. In chickens, tumors that arise from the parenchymal tissues of the thymus are among the rarer forms of neoplasia. Feldman (1936) mentioned a case reported by Saupe and described one case of his own. In the latter instance the tumor occurred in a rooster of the Barred Plymouth Rock breed, aged 2 to 3 years, that had been killed for food. The tumor occupied the ventral aspect of the cervical

region, extending from the thoracic aperture forward for a distance of 10 cm. The mass measured $10 \times 5 \times 4$ cm. and weighed approximately 250 gm. Microscopically, the tumor consisted of a richly cellular structure composed largely of intertwining sheets or bundles of polymorphic cells. A moderate number of cells were undergoing mitotic division, and in one region concentric whorls of closely packed cells resembling Hassall's corpuscles were seen. A minimal amount of lymphoid tissue was present. The structure was well supplied with blood vascular channels. Metastasis had not occurred. It was thought that the tumor arose from the reticulum cells of the thymus. Two cases of thymoma similar to the foregoing were found by Olson and Bullis (1912).

Until additional cases provide material for the study of thymic tumors in chickens, their morphologic variations and their effects on the host will remain obscure.

Carcinosarcoma. A tumor somewhat similar to that which we have designated as carcinosarcoma has been described by Jackson (1936a, b) as "carcinoma leiomyotosum." Jackson's term indicates an adenocarcinoma with a stroma of smooth muscle fibers. Jackson expressed the belief that the stroma of muscle fibers is merely a reaction on the part of the host to the growth of the neoplastic epithelial elements. Glietenberg (1927) described a similar process in the oviduct as a "myocystadenoma," implying that both muscular and epithelial elements were neoplastic. Joest and Ernesti (1915) took a similar stand with respect to 2 tumors which they diagnosed as "adenomyoma." A third mixed neoplasm with many peritoneal implants was diagnosed by them as "sarcoma carcinomatodes." Olson and Bullis (1942) found 7 cases of tumors which they termed "carcinosarcoma," all of which were widely implanted on the serosal covering of the viscera (Fig. 32.22). The epithelial elements in these cases appeared to have come from the ovary, pancreas, and adrenal. The supporting stromal tissue was considerable in amount and if

seen alone would have been considered as fibrosarcoma (Fig. 32.23). The presence of acini of epithelial cells constitutes a disquieting feature to an otherwise simple diagnosis in such cases. Bundles of smooth muscle cells also can be demonstrated in the stroma of these tumors in regions remote from where they would normally be expected. An interesting finding was a concomitant smooth muscle tumor of the mesosalpinx in 4 of the 7 cases of carcinosarcoma. However, these leiomyomas were regarded as incidental findings.

Foulds (1937) described "mixed" tumors in the course of transplantation experiments with a carcinoma of the chicken. The original tumor was believed to have arisen from the shell-secreting glands of the oviduct. In one series of transplants, bone tissue was found mixed with the carcinoma cells. Foulds discussed this feature at some length and concluded that the bone was formed either in the hyaline matrix secreted by the carcinoma cells or in the fibroblastic stroma produced by the host.

The tumors we classify as carcinosarcoma can be interpreted in different ways. They may be simple adenocarcinomas in which a highly active stromal reaction occurs and, therefore, may be considered as scirrhous adenocarcinoma. They may have originally been simple carcinomas which stimulated a stroma to the extent that it became neoplastic. On the other hand, they may have begun initially as true mixed tumors. The presence of smooth muscle tissue mixed with fibroblasts and the apparent ability of the connective tissue elements to form peritoneal implants free of the epithelial component suggest the malignant nature of the stroma. Jackson commented that such implants can be explained on the basis of assuming a primary implant of the carcinoma which became destroyed by the more rapidly growing stroma. Carcinosarcoma should not be confused with the process occasionally noted in which a connective tissue tumor infiltrates a glandular structure and causes metaplasia of the epithe-
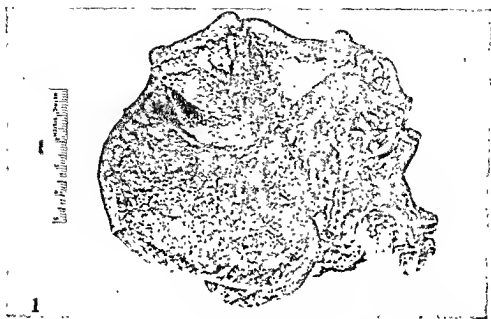
FIG. 32.22 — Carcinosarcoma showing the extensive, diffuse implantation of the neoplastic tissue over the surface of the mesentery.
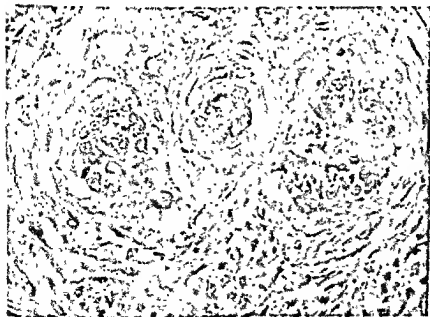
FIG. 32.23 — Carcinosarcoma. Tissue from an implant on the serosa of the mesentery. The essential features are the irregular acini of epithelial cells and the abundant stroma of immature connective tissue elements. ×600.

lial elements. Such a process might simulate a carcinosarcoma.

Although there is some confusion surrounding carcinosarcomas at this time, such neoplasms as described do seem to exist as an entity in the chicken and can be distinguished from other neoplasms. Future study should clarify their position as well as determine their histogenesis.

Teratomatous tumors. Our experience agrees with that of Hoogland (1929) that in the chicken, teratoma and teratoid tumors occur most frequently in the ovary and in the testicle. A teratoma of the ovary of a goose was recorded by Babic (1931). Grossly, those of the ovary have resembled carcinoma, and a microscopic examination has been necessary to reveal the true character of the neoplastic process. Usually the ovary has been involved extensively, and secondary foci representing implantation metastatic growths were commonly present. The propensity for ovarian teratoma to produce cysts containing gelatinous or mucoid material was well illustrated in several of our cases. In two of the six cases in our collection, metastatic foci had been established in the lungs.

The microscopic appearance of these tumors is of some interest. A brief summary of the histologic characteristics of the six cases mentioned follows:

Case 1. The growth consisted of a mixture of (1) squamous epithelium; (2) columnar epithelium, with and without mucin; and (3) primitive mesodermal cells, with definite sarcomatous changes. Metastasis to the lung, largely of columnar epithelium, had occurred.

Case 2. Much of the tissue had an embryonic appearance. The tumor consisted of (1) large mucus-forming epithelial cells, which were inclined to form cysts containing mucus; (2) squamous epithelium with central areas of keratin; and (3) large amounts of sarcomatous tissue of the histiocytic variety in which mitosis was common. The pulmonary metastasis was predominantly sarcomatous.

Case 3. The mass was quite cystic, and

the following varieties of epithelium were discernible: (1) squamous, (2) high columnar, (3) low columnar, and (4) epithelium composed of mucus-containing cells.

Case 4. The tumor was epithelial in character and contained squamous cells and tall columnar and low columnar cells.

Case 5. This tumor was a mixture of epithelial and undifferentiated sarcomatous elements. Epithelial cells with and without mucus and with cilia were demonstrated.

Case 6. There were present nests of squamous epithelial cells and regions of papillary adenocarcinoma.

In the six cases mentioned, the occurrence of squamous epithelium with the formation of central regions of keratin was the most characteristic feature (Fig. 32.24). The mesodermal elements frequently exhibited a bizarre picture, with undifferentiation a notable feature. In the ovary as well as in the testicle, cells occur which have the inherent possibility of producing tissues representing all three embryonic layers. From such elements, complex, jumbled, tumorlike conditions may arise that may represent an attempt to produce an embryo. However, in our material none of the ovarian teratomas contained tissue representing the entoderm; all were didermic in character. Apparently in the chicken tridermic teratomas are of rare occurrence.

We have observed one didermic teratoma of the testicle, and Jackson (1956a) mentioned a tridermic teratoma of the testicle of a rooster. Our specimen was apparently similar in many respects to that described by Olson and Bullis except that we did not find any cartilage in ours. A few irregular islands of squamous epithelium were also discernible in the midst of mesodermal elements. Babic described two cases of teratoma of the testis in chickens.

The teratomas of the testicle may attain considerable size. The specimen described by Olson and Bullis measured $13 \times 10 \times 7$ cm. and weighed 606 gm. (Fig. 32.25).

FIG 32.24.—Didermic teratoma of the ovary of a 1-year-old chicken. Note several epithelial "pearls" surrounded by sarcomatous stroma. ×120.

In a teratoma that probably arose in the left testicle of a 21-month-old rooster described by Sheather (1911), the mass measured 15 × 11.5 × 7.5 cm. and weighed 500 gm. These tumors are usually well encapsulated and have an irregular

FIG. 32.25.—Teratoma of the testis. A small nodule of normal testicular tissue extends from the lower aspect of the tumor.

nodulated surface. The color varies from grayish-white to dark livid. Necrosis is common, and cysts of variable sizes are likely to be present. Pohl described what he designated adenocarcinoma of the testicle of a rooster that we believe was probably a teratoma.

Teratoma of the testis has been produced experimentally in chickens by Michalowski (Bagg, 1936), Bagg (1936), and Falin and Gromzewa (1940). This has been accomplished by repeated intratesticular injections of small quantities of zinc salts (chloride, sulfate, and nitrate). An associated seasonal factor is present in that teratomas of the testis can be produced only during early spring. This factor may be affected by administration of *gonadotropic hormone*. Baker (*Fowlds*, 1931) succeeded in making two successful transplants of a spontaneous ovarian teratoma of the chicken.

Embryonal nephroma. This tumor, which we have classified perhaps more or less arbitrarily with the mixed tumors, usually originates in the kidney and is one of the commoner neoplasms of chickens. Among the terms applied to this neoplasm are adenoma, cystadenoma, adenomyosarcoma, sarcocarcinoma, sarco-adenoma,

rhabdomyo-adenosarcoma, adenosarcoma, and Wilms' tumor.

Since it is generally agreed that these tumors arise from primitive nephrogenic tissue, a term that indicates their origin rather than one which may be descriptive in a morphologic sense would seem desirable. For this reason we believe that these tumors, regardless of the variations of their structural pattern, should be designated embryonal nephroma.

Histogenesis. It seems a reasonable presumption that this tumor, which is characterized by more or less unpredictable structural variations, has its origin, regardless of anatomic differences that may occur in different specimens, in a multipotent type of nephrogenic cell. Such a cell possesses all the capacity for differentiation inherent in an immature mesodermal cell

While the vast majority of embryonal nephromas occur in the substance of the kidney, these tumors may arise anteriorly or posteriorly, separate and removed from the nephric tissue (Feldman, 1930). The origin of embryonal nephromas in extranephric situations may be explained on the basis of the failure of certain portions of the mesonephron to be utilized in the development of the permanent kidney. These remains of the Wolffian body, instead of retrogressing, continue to grow but without the guiding influences characteristic of normal tissues, and a neoplastic process results.

Frequency of occurrence. The relative incidence of the occurrence of embryonal nephroma in chickens is uncertain. However, it is generally believed that it occurs more commonly in chickens than in mammals. Reliable statistical data on the incidence of embryonal nephroma are meager. Among a total of 113 chicken tumors exclusive of those designated "leukotic tumors" reported by Goss (1940a), 4 were considered embryonal nephromas. In Jackson's (1936a, p. 418) series of avian neoplasms, the embryonal nephromas accounted for 3.5 per cent of the total. Olson and Bullis (1942) found 21 (5.5 per cent)

embryonal nephromas among 384 tumors.

There is no evidence that one sex is more susceptible to the development of embryonal nephroma than the other. Although the majority of these tumors are discovered in chickens during the young adult or adult stage of life, their histogenesis provides possibilities for their early development. In fact, it is likely that in many if not most instances these tumors are present and of demonstrable proportion when the chick is hatched. Whether or not breed is of significance in the occurrence of embryonal nephroma is problematic. Mathews (1929a) expressed the opinion that the Barred Plymouth Rock breed shows a particular predisposition for the development of this tumor.

Sites of occurrence. Most embryonal nephromas are found embedded in or arising from the substance of the kidney, although occasionally a specimen is encountered that has no direct contact with this organ. The tumors are usually unilateral. In 31 of a series of 41 cases in our collection, one kidney only was affected, and in 10 cases both kidneys were affected. Although there has been an impression that the left kidney is involved most often, in our material of 31 unilateral embryonal nephromas, 15 occurred in the right kidney, 14 occurred in the left kidney, and in 2 instances the specific kidney affected is not known. Jackson (1936a), however, mentioned that in 4 specimens in his collection in which the site of occurrence was known, 3 arose from the left kidney and 1 from the right kidney.

Effects on the host. As with many other neoplasms of chickens, it is difficult to determine what the effect of the presence of an embryonal nephroma may be on the well-being of the animal. Since in the chicken embryonal nephroma seldom if ever gives rise to recognizable objective symptoms, one must conclude that if only one kidney is involved and if metastasis has not occurred, the presence of a tumor of this kind is not likely to be a serious physiologic handicap. Exception must of

course be taken in those instances in which the tumor is unusually large. Specimens of such size as to occupy a considerable portion of the abdominal cavity must provide a source of discomfort to the host. Paralysis may result if the tumor causes pressure on the nerves to the leg. If large hemorrhagic cysts should rupture or if there are extensive regions of necrosis, secondary disturbances may develop. If metastasis should occur, the usual effect of malignant lesions may be expected. If both kidneys are affected and the neoplastic process is extensive and progressive, it is conceivable that sufficient nephric tissue may be destroyed to prevent adequate secretion of urine.

Gross and microscopic description. The gross appearance of embryonal nephroma is subject to considerable variation depending on the size and anatomic situation. The size varies within wide limits from a small focus of grayish-pink tissue embedded in the substance of the kidney to huge lobulated masses that have replaced nearly all of the nephric tissue. One of our specimens had an extranephric origin, weighed approximately 200 gm., and measured 7 × 6 × 6 cm. An exceptionally large keratinizing embryonal nephroma in a 5-month-old Barred Rock cockerel was observed at the Ontario Veterinary College (1945). Two tumors were present—one large and one small. The size of the larger specimen was noteworthy. It meas-

ured 15 × 10.5 × 10 cm., and weighed 802 gm. The color may be yellow, grayish-white, or grayish-pink, and while most specimens have a firm fleshlike consistency, small to large cysts and foci of necrosis may provide regions that are soft and spongelike. Small, hard, pearly nodules may be recognized in some specimens (Fig. 32.26). Such dense material was found in abundance in a large keratinizing embryonal nephroma which we described some years ago (Feldman and Olson, 1933).

Although the contour of these tumors is usually irregular and frequently interrupted by fissures of variable depths, the surface of the nonkeratinizing varieties is smooth and glistening. The large keratinizing specimen previously mentioned was quite roughened, owing to the presence of innumerable closely packed, grayish-yellow, kernel-like units of variable sizes. These gave the surface of the tumor a strikingly pebbled or bosselated appearance.

The tumors are usually well supplied with blood vessels, and in large specimens diffuse regions of hemorrhage may be present. When the interior of moderately large to large specimens is exposed by incision, the distinct lobulations are often apparent. Cysts, if present, may contain a semiclear or hemorrhagic fluid.

The multipotent embryonic character of the elements which give rise to embryonal

FIG. 32.26 — Keratinizing embryonal nephroma of the left kidney. Conglomerate masses of hard "pearly" nodules may be seen.

nephromas provides for the widest possible variation of their microscopic appearance. The structure may be relatively simple or confusingly complex even within the substance of the same tumor. The most consistent feature is the great variability of their histologic appearance. No two are alike. It is difficult, therefore, to compose a written description of the microscopic features of a "typical" embryonal nephroma. In cases in which the disease is represented by the occurrence of a tiny focus just visible grossly, the essential morphologic features consist of a compact, more or less diffuse region of undifferentiated spherical or oval cells situated in the cortical zone of the kidney. Encapsulation is not evident, and the neoplastic cells at the periphery of the lesion are infiltrating into the surrounding substance of the kidney. In large specimens in which it may be presumed that the process has existed for a long period, the microscopic picture is more bizarre. The parenchyma of the tumor is highly cellular, and irregular septa of connective tissue dividing the tumor into indistinct lobules may or may not occur. The parenchyma of the process usually presents tubules or ductlike structures (Fig 32.27)

Frequently, solid nests of cuboidal cells arranged in an acinar fashion may predominate. The ductlike or tubulelike spaces are lined by single or several layers of cuboidal or columnar cells which apparently arise from the surrounding undifferentiated elements.

In some regions the undifferentiated elements, which may be cuboidal or spindle shaped, constitute the predominant feature of the histologic picture. In other regions epithelial elements as represented by the adenomatous character of the structure are the most striking feature. Since the structure of the majority of these tumors represents a mixture of undifferentiated and differentiated cells, no useful purpose is served by attempting to determine in a given specimen which of these elements predominates.

Although the tubular spaces in many of these tumors when examined microscopically are apparently empty, in some a mucinlike substance that is probably the product of the cells lining the tubules occurs. If this substance is produced in excessive amounts, large cysts are formed. Striking evidence of the multipotent potentialities of the cells which give rise to embryonal nephroma may be found in

FIG. 32.27 — Embryonal nephroma, kidney of a chicken. Epithelial elements predominate. ×150.

specimens that contain, in addition to the undifferentiated sarcomatous elements, typical hyaline cartilage and bone. We have not recognized striated muscle in our material, although this tissue could conceivably occur.

Fairly frequently, embryonal nephromas of the chicken are encountered in which epithelial elements reveal a marked epidermoid differentiation. This occurred in varying degrees in 6 of 20 cases we have studied. These changes may consist of small foci of rather typical squamous cells resembling in every respect epithelial "pearls." An occasional embryonal nephroma may occur in which keratinization is a predominant feature.

The vascular supply of the tumors is usually considerable, and hemorrhagic extravasations with organizing or organized blood clots may be present. Edema is present sometimes among the stromal elements, and a few to large numbers of eosinophilic granulocytes may occur promiscuously throughout the tumor.

Metastasis. Although mitosis and other evidences of instability and progression are commonly observed in embryonal nephroma, metastatic dissemination of these tumors appears to be relatively infrequent. Metastasis has been recorded by Jackson (1936a), by Mathews (1929a), and by Duran-Reynals (1946b), but has not been demonstrated in the material at our disposal. In our experience most of the larger specimens have been rather well encapsulated, and while infiltration of the neoplastic elements into the substance of the kidney occasionally occurs, this is infrequent. As a matter of fact, the fibrous zone of connective tissue that is usually present between the neoplastic and the nephric elements must be considered a formidable barrier against the invasive tendencies of the tumor. The destructive effect on the kidney of many embryonal nephromas is due to the pressure atrophy and other retrograde influences that ensue as a consequence of the gradual, progressive expansion of the growth. It should be kept in mind, however, that these are at

least locally malignant tumors, and most if not all of these should be capable of setting up metastatic foci if circumstances are propitious.

Transplantability. Duran-Reynals (1946b) attempted transplants from 10 spontaneous cases, 7 of which proved nontransplantable. One yielded a fibroma type of growth, and grafts from another resembled the original embryonal nephroma with sarcomatous features. Neither of these could be carried in serial passage. The third transplantable embryonal nephroma was carried in regular serial passage as a sarcoma. Duran-Reynals and Shrigley (1946) are inclined to regard this as an independent sarcoma since a cell-free etiological agent was demonstrated.

The renal adenocarcinoma produced by the ES-4 leukosis virus was mentioned in the section on fowl leukosis (Carr, 1956). Ishiguro *et al.* (1962) and Heine *et al.* (1962) have studied the nephroblastomas produced by the BAI myeloblastosis virus in considerable detail and consider it to closely resemble the Wilms' tumor since they contain all the elements of the embryonal nephroma.

Diagnostic characteristics. In chickens, as Jackson (1936a, p. 351) pointed out, embryonal nephroma should always be considered when a tumor occurs in or near the kidney or when a tumor is encountered suspended from the lumbar region although somewhat removed from the kidney. Microscopically, these tumors are usually entirely unlike any other neoplasms. The mixture of undifferentiated cuboidal or spindle cells and of adenomatous epithelial structures, keratin, cartilage, or bone should be sufficient for their correct identification.

CONCOMITANT NEOPLASIA

Concomitant or multiple neoplasia, in which two or more distinct types of neoplastic disease occur in the same bird, appears to have received little attention in the past. Although multiple neoplasia has been noted in other animals, especially old dogs (Feldman, 1932), only a few com-

ments were found in the literature on concomitant neoplasia of the chicken. Perhaps the fact that relatively few chickens are allowed to attain advanced age may be a factor in the apparent infrequency of multiple neoplasia. Another explanation for the relatively few observed cases is that they are not recognized since relatively few diagnoses of spontaneous avian neoplasia are made with the aid of histologic examination. Thorough histologic examination is obviously necessary for the recognition of instances of concomitant neoplasia, for without such an examination, unexamined foci of tumors may be assumed to represent metastasis of a primary growth.

Jackson (1936a) discussed "collision" tumors in which elements of two distinctly different neoplastic processes become intermingled to create the impression of a "mixed" tumor. He described an instance of a histiocytic sarcoma and myelocytoma occurring in the same chicken. Babic (1931) described a chicken which had a fibroma of the intestine in addition to hemangioma of the liver, kidney, and serosa of the proventriculus. Jármai (1939) found both fowl leukosis and fibrosarcoma as concomitant tumors in a parakeet.

Olson and Bullis (1942) observed 19 instances of concomitant neoplasia in a collection of 384 neoplasms found in 365 chickens. Of 6 chickens that had lymphocytoma, 4 also had embryonal nephroma, 1 had neurogenic sarcoma, and 1 had leukosis. Of 5 chickens that had fowl leukosis, 2 also had myelocytoma, and 1 had fibrosarcoma. Of 6 birds that had leiomyoma, 4 were also affected with carcinosarcoma, and 2 with carcinoma. Myelocytoma and embryonal nephroma were found in 1 chicken. Histiocytic sarcoma and hemangioma were concomitant tumors in 1 chicken; adenoma of the thyroid and melanoma of the tongue, in another; and a third chicken had an adenoma of the pancreas and a fibrosarcoma in the pelvic cavity.

The possibility of an etiologic relation between neoplasms occurring simultaneously in the same chicken is suggested in cases of concomitant neoplasia. Experimentally, several strains of the agent responsible for fowl leukosis have been shown to be capable of inducing fibrosarcoma. The spontaneous occurrence of this combination of concomitant neoplasia, therefore, may not be entirely due to chance.

Since certain types of spontaneous tumors are relatively common in the chicken, the occurrence of the more common varieties in birds of a group under experimental observation may be anticipated. This probability necessitates extensive and careful provision for the control of experiments designed to attempt transmission of neoplasia in chickens. Concomitant neoplasia may occur in such experimental birds. Usually such cases should be interpreted as a spontaneous neoplasm which occurred in a bird in which a second type of neoplasia also had developed as the result of experimental inoculation. An example is the association of lymphocytoma and fowl leukosis in a chicken which had received material containing the causative agent of fowl leukosis. Olson (1936) observed two such instances and regarded the lymphocytoma as of spontaneous origin and unrelated to the fowl leukosis.

Concomitant neoplasia may develop in experimental birds treated with carcinogenic agents. Murphy and Sturm (1941a) reported the finding of leukemia and adenocarcinoma in a chicken that had received intramuscular injections of dibenzanthracene in lard. The dibenzanthracene was regarded as responsible for the development of both neoplastic processes.

TUMORS OF BIRDS OTHER THAN CHICKENS

Various types of neoplastic disease have been reported from different species of both wild and domesticated birds. Although adequate data are not available to make absolute comparisons, in no other

single species of bird do tumors appear as likely to develop as in the domestic chicken.

The observations at the University of Leipzig from 1899 to 1931 indicate the relative incidence of neoplasia among *various domesticated species of birds* (Eber and Malke, 1932). During this period 2,353 pigeons were submitted to necropsy, and 14 were affected with neoplastic disease, an incidence of 0.6 per cent. Of 720 geese examined, 1 had "chondrosarcoma myxomatodes." Among 692 ducks, 1 had adenocarcinoma of the liver; among 459 turkeys examined, 2 had neoplasms of the liver (mixed cell type of sarcoma); and among 204 pheasants, 1 had adenoma of the lung, and 1 had myxosarcoma of the liver. In the same interval of time 53 guinea fowl, 52 peacocks, and 25 swans were examined, in none of which was neoplastic disease found. A total of 11,903 chickens were examined, and 3.12 per cent (371) had tumors. Babic (1931) in Yugoslavia reported 16 cases of neoplasia in birds other than chickens. These were encountered during the period between 1925 and 1931. In this interval 59 pigeons were examined, 5 of which had tumors; 27 parakeets, 3 of which had tumors; 45 canaries, 1 of which had a tumor; 40 geese, 2 of which had tumors; 29 turkeys, 2 of which had tumors; 10 pheasants, 1

of which had a tumor; 14 owls, of which 1 had a tumor; 2 storks, of which 1 had a tumor. Of 81 ducks examined, none revealed the presence of neoplasia.

Fox (1923) listed 44 neoplasms found in captive wild birds. Eleven varieties of *tumor were identified.* Species most commonly affected were in the family of Psittacidae and accounted for 23 of the cases, 16 of which were in the undulated grass parakeet *(Melopsittacus undulatus).* It is of interest to note that in a bird of this species Jármai (1939) found a sarcoma and fowl leukosis, and Schlumberger (1954) has reported over 100 spontaneous pituitary tumors in parakeets. Epidermoid carcinomas of the feet of wild birds were reported by Emmel (1930).

Neoplasms in other species of birds are quite similar in general character to those found in chickens. Most reports in the literature concern cases of epithelioblastoma and tumors of connective tissue. Apparently lymphocytoma may occur to a significant extent in turkeys. Simpson *et al.* (1957) report the disease in 60 of a flock of 1,200 8-month-old turkeys. Most of these were nodular in character suggesting partial resistance of the host. Neoplasia of the lymphoid cell system may be encountered occasionally in various species of birds but in none is it as common as in the chicken.

REFERENCES

Abels, H.: 1929. Die Geschwülste der Vogelhaut. Zeitschr. f. Krebsforsch. 29,183.

Apperly, F. L.: 1933. Primary carcinoma of the lung in the domestic fowl. Am. Jour. Cancer 23:556.

Ask-Upmark, E.: 1938. Ein gehäuftes ("epidemisches") Vorkommen von Hühnertumoren. Frankfurt. Zeitschr. f. Path. 52:51.

Atanasiu, P.: 1952. Les granuloblastoses chez l'embryon de poulet après inoculation de virus érythroblastique. Aspects hématologiques. Sang 23:325.

——: 1956. Hépatite spontanée à virus associée chez l'embryon de poulet à la leucose érythroblastique. Compt. Rend. Acad. Sci. 243:719.

Babic, I.: 1931. Spontani tumori perad. Veterinarski Arhiv. 1:158.

Bagg, H. J.: 1936. Experimental production of teratoma testis in the fowl. Am. Jour. Cancer 26:69.

Baker, S. L.: Quoted by Foulds, 1934.

Ball, R. F.: 1945. Two unusual neoplasms in the chicken iris. Cornell Vet. 35:585.

Barber, C. W.: 1942. The effect of the rearing environment upon the incidence of avian leukosis complex. Cornell Vet. 32:194.

——: 1943. The effects of environment on the incidence of avian leukosis complex lesions among resistant and non-resistant chickens. Cornell Vet. 33:78.

Beard, D., Beaudreau, G. S., Bonar, R. A., Sharp, D. G., and Beard, J. W.: 1957. Virus of avian

erythroblastosis. III. Antigenic constitution and relation to the agent of avian myeloblastosis. Jour. Nat. Cancer Inst. 18.231.

Beard, J. W.: 1956. Virus of avian myeloblastic leukosis Poultry Sci. 35:203.

Berner, O : 1923. Virslisme surrénal chez une poule. Rev. franc. d'endocrinol. 1:474.

Boyd, W.: 1938   A Text book of Pathology: An Introduction to Medicine. Lea and Febiger, Philadelphia, pp 42-45, 320-25.

Brewer, N. R., and Brownstein, B.: 1946. The transmission of lymphomatosis in the fowl. Am. Jour. Vet. Res 7:123.

Bryan, W. R.: 1958. Analysis of the possible role of inhibitors in masking and latency of tumor viruses Symposium on Latency and Masking in Viral and Rickettsial Infections. Burgess Publishing Co., Minneapolis.

Burmester, B. R.: 1945. The incidence of lymphomatosis among male and female chickens Poultry Sci. 24:569.
———: 1947. Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell free preparations. Cancer Res. 7:786.
———: 1952. Studies on fowl lymphomatosis. Ann. N.Y. Acad. Sci. 54:992.
———: 1956. The shedding of the virus of visceral lymphomatosis in the saliva and feces of individual normal and lymphomatous chickens. Poultry Sci. 35:1089.
———, and Belding, T. C.: 1947. Immunity and cross immunity reactions obtained with several avian lymphoid tumor strains. Am. Jour Vet. Res. 8:128.
———, Fontes, A. K., and Walter, W. G.: 1960. Contact transmission of Rous sarcoma. Jour. Nat. Cancer Inst. 25:307.
———, and Gentry, R. F.: 1956 The response of susceptible chickens to graded doses of the virus of visceral lymphomatosis Poultry Sci. 35:17.
———, Gentry, R. F., and Waters, N. F.: 1955. The presence of the virus of visceral lymphomatosis in embryonated eggs of normal appearing hens. Poultry Sci. 34 609.
———, Gross, M. A., Walter, W. G., and Fontes, A. K.: 1959. Pathogenicity of a viral strain (RPL 12) causing avian visceral lymphomatosis and related neoplasms. II. Host-virus interrelations affecting response. Jour Nat Cancer Inst. 22:103.
———, and Nelson, N. M.: 1945. The effect of castration and sex hormones upon the incidence of lymphomatosis in chickens. Poultry Sci 24:509.
———, and Prickett, C O.: 1945 The development of highly malignant tumor strains from naturally occurring avian lymphomatosis. Cancer Res. 5:652.
———, and Walter, W. G.: 1961. Occurrence of visceral lymphomatosis in chickens inoculated with Rous sarcoma virus Jour. Nat. Cancer Inst. 26(3).511.
———, Walter, W. G., and Fontes, A. K.: 1957. The immunological response of chickens after treatment with avian vaccines of visceral lymphomatosis. Poultry Sci. 36.79.
———, and Waters, N. F.: 1955. The role of the infected egg in the transmission of visceral lymphomatosis Poultry Sci. 34-1445.
———, and Waters, N F.: 1956. Variation in the presence of the virus of visceral lymphomatosis in the eggs of the same hens. Poultry Sci. 35:989

Campbell, J. G.: 1945   Histocytic and fibroblastic sarcoma (mixed-cell sarcoma) in the domestic fowl. Jour. Comp Path. and Therap. 55:323.
———: 1945. Neoplastic disease of the fowl with special reference to its history, incidence, and seasonal variation. Jour. Comp. Path. and Therap. 55:308.
———: 1949. Spontaneous hepatocellular and cholangiocellular carcinoma in the duck. An experimental study. Brit. Jour. Cancer 3:198.
———: 1955. Induction of multiple primary tumours in fowls with 2 acetamidofluorene. Brit. Jour. Cancer 9:163.

Carr, J. G · 1943. Prolonged antibody production following recovery of fowls from Rous No. 1 sarcoma. Brit. Jour. Exper. Path. 24:138.
———: 1944. Lack of transmission of avian tumor virus from carrier hens to their offspring via the egg. Proc Roy. Soc. Edinburgh, Sec B, 62:54.
———: 1956. Renal adenocarcinoma induced by fowl leukemia virus. Brit. Jour. Cancer 10:379.

Claude, A., and Murphy, J. B.: 1933. Transmissible tumors of the fowl. Physiol. Rev. 13.246.

Cole, R. K.: 1916. An avian reuroblastoma. Cornell Vet. 36:350.
———, and Hutt, F B · 1951. Evidence that eggs do not transmit leukosis. Poultry Sci. 30.205.

Curtis, M R 1916. Frequency of occurrence of tumors in the domestic fowl. Jour. Agr. Res. 5.397.

Darcel, C. le Q.: 1957. Research on the fowl leucoses — an abridged review. Canad. Jour. Comp. Med. and Vet. Sci 21:344.
———, and Franks, L. M. 1955 Angiomatoid lesions of the skin in young chicks. Jour. Path. and Bact. 69:499.

Davis, O. S., and Doyle, L. P.: 1947a. Studies in avian leucosis. I. The transmissibility of visceral lymphomatosis. Am. Jour. Vet. Res. 8:103.
———, and Doyle, L. P.: 1947b. Studies in avian leucosis. II. The use of biopsy technique in the study of viral lymphomatosis. Am. Jour. Vet. Res. 8:113.

Duran-Reynals, F.: 1940. Neuralization of tumor viruses by the blood of normal fowls of different ages. Yale Jour. Biol. Med. 13.61.

———: 1946a. Transplantability and presence of virus in spontaneous sarcomas and fibromas of chickens in relation to the age of the tumor-bearing animal. Cancer Res. 6:529.

Duran-Reynals, F.: 1946b. On the transplantability of lymphoid tumors, spontaneous and nephromas, and carcinomas of chickens. Cancer Res. 6:545.

———: 1950. The significance of the non-neoplastic lesions induced in the central nervous system of ducklings by the virus of a duck variant of the Rous sarcoma. Yale. Jour. Biol. and Med. 22:555.

———, Burmester, B. R., Cottrall, C. E., and Bryan, E.: 1953. Studies on the origin of the naturally occurring antibodies against tumor viruses developing in aging chickens. Cancer Res. 13:408.

———, and Shrigley, E. W.: 1946. A study of five transplantable chicken sarcomas induced by viruses. Cancer Res. 6:535.

Eber, A., and Krieghaum, A.: 1916. Untersuchungen über Eiersacks- und Eileitergeschwulste beim Haushuhn. Zeitschr. f. Krebsforsch. 15:404.

———, and Malke, E.: 1932. Geschwulstbildungen beim Hausgeflügel. Zeitschr. f. Krebsforsch. 36.178.

Ehrenreich, M., and Michaelis, L.: 1906. Über Tumoren bei Hühnern. Zeitschr. f. Krebsforsch. 4:586.

Ellermann, V.: 1920. Histogenese der übertragbaren Hühnerleukose. I. Die myeloische Leukose. Folia haemat. 26:135.

———, and Bang, O.: 1908. Experimentelle Leukämie bei Hühnern. Zentralbl. f. Bakt. I. Orig. 46:595.

Elsner: Quoted by Reis and Nóbrega, 1955a.

Emmel, M. W.: 1930. Epidermoid cancers on the feet of wild birds. Jour. Am. Vet. Med. Assn. 77.641.

Engelbreth-Holm, J.: 1942. Spontaneous and Experimental Leukaemia in Animals. Oliver and Boyd, London, 245 pp.

———, and Rothe Meyer, A.: 1932. I. Bericht über neue Erfahrungen mit einem Stamm Hühner-Erythroleukose. Acta Path. et microbiol. Scandinav. 9.293.

———, and Rothe Meyer, A.: 1935. On the connection between erythroblastosis (haemocytoblastosis), myelosis, and sarcoma in chicken. Acta Path. et Microbiol. Scandinav. 12:352.

Epstein, M. A.: 1957. Observations on the Rous virus; fine structure and relation to cytoplasmic vacuoles. Brit. Jour. Cancer 11.268.

Erichsen, S., and Harboe, A.: 1953. So-called gliomas observed in chickens with toxoplasms. Acta Path. et Microbiol. Scandinav. 33:581.

Ewing, J.: 1928. Neoplastic Diseases; A Treatise on Tumors. Third Ed. W. B. Saunders and Co., Philadelphia, Chap. 16, pp. 240-56.

Falin, L. I., and Gromzewa, E. Z.: 1940. Zur Pathogenese der experimentellen teratoiden Geschwulste der Geschlechtsdrüsen. II. Mitteilung. Teratoide Geschwulste der Geschlechtsdrüsen bei Hühnen, erzeugt durch Injektionen von Zn (NO₃)₂ Lösung. Virchows Arch. f. path. Anat. 306.578. Abst. Cancer Res. 1:580. (1941.)

Feldman, W. H.: 1930. Extranephric embryonal nephroma in a hog. Jour. Cancer Res. 14:116.

———: 1932. Neoplasms of Domesticated Animals. W. B. Saunders Co., Philadelphia, pp. 51-53; 178-93; 357.

———: 1936. Thymoma in a chicken (Gallus domesticus). Am. Jour. Cancer 26.576.

———, and Olson, C., Jr.: 1933. Keratinizing embryonal nephroma of the kidneys of the chicken Am. Jour. Cancer 19:47.

Fischel, A.: 1922. Zur Eröffnung des neuen Institutes für Embryologie. Wien. klin. Wochenschr. 35:355.

Foulds, L.: 1934. The filtrable tumours of fowls. A critical review. Suppl. to 11th Scientific Rep. of Cancer Res. Fund, London, 41 pp.

———: 1937. A transplantable carcinoma of a domestic fowl, with a discussion of the histogenesis of mixed tumors. Jour. Path. and Bact. 44:1.

Fox, H.: 1923. Disease in Captive Wild Mammals and Birds. J. B. Lippincott Co., Philadelphia, pp. 402-82.

Friedgood, H. B., and Uotila, U. U.: 1941. Occurrence of ovarian "tumors" in spontaneous virilism of the hen. Endocrinology 29.47.

Furth, J.: 1931. Erythroleukosis and the anemias of the fowl. Arch. Path. 12:1.

———: 1933. Lymphomatosis, myelomatosis, and endotheliomia of chickens caused by a filtrable agent. I. Transmission experiments. Jour. Exper. Med. 58.253.

———: 1935. Lymphomatosis in relation to fowl paralysis. Arch. Path. 20.379.

Glürentjerg: 1927. Ein Myokystadenom des Eileiters eines Huhnes. Zeitschr. f. Fleisch. u. Milchhyg. 37:155.

Goldberg, S. A.: 1919. The differential features between melanosis and melanosarcomas. Jour. Am. Vet Med. Assn. 56:140-53; 250-64.

Goss, L.: 1940a. The incidence and classification of avian tumors. Rep. N.Y. St. Vet. Coll (1939-40), p. 103.

———: 1940b. The incidence and classification of avian tumors. Cornell Vet. 30:75.

Hamilton, C. M., and Sawyer, C. E.: 1939. Transmission of erythroleukosis in young chickens. Poultry Sci. 18:588.

Harris, R. J. C.: 1955. Acquired tolerance in turkeys to the Rous sarcoma agent. Brit. Emp. Cancer Campaign, 33rd Ann. Rep. 65.

Heine, F.: 1931. Hühnergeschwülste. Zeitschr. f. Krebsforsch. 33:76.

Heine, U., de Thé, G., Ishiguro, H., Sommer, J. R., Beard, D., and Beard, J. W.: 1962. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. II. Nephroblastoma (Wilms' tumor): Ultrastructure. Jour. U.S. Nat. Cancer Inst. 29:41.

Hoogland, H. J. M.: 1929. In van Heelsbergen, T., Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart, pp. 484-97.

Hutt, F. B., and Cole, R. K.: 1947. Genetic control of lymphomatosis in the fowl. Science 106:379.

———, Cole, R. K., and Bruckner, J. H.: 1941. Four generations of Iowa bred for resistance to neoplasms. Poultry Sci. 20:514.

Ishiguro, H., Beard, D., Sommer, J. R., Heine, U., de Thé, G., and Beard, J. W.: 1962. Multiplicity of cell response to the BAI strain A (myeloblastosis) avian tumor virus. I. Nephroblastoma (Wilms' tumor): Gross and microscopic pathology. Jour. Nat. Cancer Inst. 29:1.

Jackson, C.: 1936a. The incidence and pathology of tumours of domesticated animals in South Africa: A study of the Onderstepoort collection of neoplasms with special reference to their histopathology. Onderstepoort Jour. Vet. Sci. 6:1.

———: 1936b. Neoplastic diseases in poultry. Jour. So. African Vet. Med. Assn. 7:99.

———: 1954. Gliomas of the domestic fowl. Onderstepoort Jour. Vet. Res. 26:501.

Jármai, K.: 1935. Tumorerzeugung mit dem Leukoseagens der Hühner. Arch., f. wiss. u. prakt. Tierheilk. 69:273.

———: 1938. Über die Virusfraktionen bei der übertragbaren Hühnerleukose. Arch. f. wiss. u. prakt. Tierheilk. 73:295.

———: 1939. Leukose und Sarkom beim Weitensittich. Arch. f. wiss. prakt. Tierheilk. 74:316.

Joest, E., and Ernesti, S.: 1915–1916. Untersuchungen über spontane Geschwülste bei Vögeln mit besonderer Berücksichtigung des Haushuhns. Zeitschr. f. Krebsforsch. 15:1.

Johnson, E. P.: 1934. The etiology and histogenesis of leucosis and lymphomatosis of fowls. Va. Agr. Exper. Sta. Tech. Bul. 56.

Jordan, H. E.: 1936. The relation of lymphoid tissue to the process of blood production in avian bone marrow. Am. Jour. Anat. 59:249.

Junghere, E., and Wolf, A.: 1939 Gliomas in animals, a report of two astrocytomas in the common fowl. Am. Jour. Cancer 37:493.

Kenny, S. G.: 1955. Studies in avian neoplasia III. Incidence of Rous virus neutralizing antibodies and lymphomatosis in chickens inoculated with neoplastic and normal tissue suspensions. Am. Jour. Vet. Res. 14:249.

———, McClary, C. F., and Zander, D. V.: 1961. Further observations concerning Rous virus neutralizing antibodies and neoplasia. fl In breeder and experimental flocks. Avian Dis. 5:337.

———, and Neuill, P. V.: 1953. Studies in avian neoplasia. II. Incidence of Rous virus neutralizing antibodies in serums collected from flocks experiencing losses due to lymphomatosis. Am. Jour. Vet. Res. 14:123.

Lichtenstein, B. W.: 1919 A Textbook of Neuro pathology. W. B. Saunders Co., Philadelphia, pp. 474.

Love, R., and Sharpless, G. R.: 1954. Studies on a transplantable chicken tumor (RPL 12 lymphoma). II. Mechanism of regression following infection with an oncolytic virus. Cancer Res. 14:640

Lucas, A. M., Denington, E. M., Cottral, G. E., and Burtmeister, B. R.: 1954. Production of socalled normal lymphoid foci following inoculation with lymphoid tumor filtrate. I. Pancreas 2 Liver and spleen. Poultry Sci. 33:562 and 571.

———, and Oakburg, E. F.: 1950 Lymphoid tissue and its relation to so called normal lymphoid loci and to lymphomatosis. II. Quantitative analysis of lymphoid areas in the pancreas of laboratory and farm chickens. Am. Jour. Path. 26:75.

McGowan, J. P.: 1928. On Rous. Leucose and Allied Tumours in the Fowl: A Study in Malignancy. The Macmillan Co. New York.

Marine, D., and Rosen, S. H.: 1910. Increase in the incidence of lymphomatosis in male fowls by castration. Am. Jour. Cancer 39:515.

———, and Rosen, S. H.: 1941. Sex hormones and lymphomatosis in fowls. Proc. Soc. Exper. Biol. and Med. 47:61.

Mathews, F. P.: 1929a Adenosarcomata of the kidneys of chickens. Jour. Am. Vet. Med. Assn. 74:553.

———: 1929b. Leukochloroma in the common fowl. Its relation to myelogenic leukemia and its analogies to chloroma in man Arch. Path. 7:412

———, and Walkey, F. L.: 1930 Hypernephromas in the common fowl. Jour. Am. Vet. Med. Assn. 77:218.

Meyer: Quoted by Feldman, W. H. (1932.)

Michalowsky, I. O.: Quoted by Bagg. H. J

Monius, W. S., and Delaplane, J P 1952 Hemangiomas in the skin of the chicken. Cornell Vet. 42:193.

Murphy, J. B., and Sturm, E.: 1941a. Further investigation of induced tumors in fowls. Cancer Res. 1:477.

————, and Sturm, E.: 1941b. Further investigation on transmission of induced tumors in fowls. Cancer Res. 1 609.

Nelson, N. M.: 1946. Leiomyoma of the ventral ligament of the oviduct in the chicken. Am. Jour. Path. 22:1047.

Newberne, P. M., Carlton, W. W., and Wogan, G. N.: 1964. *Hepatomas in rats and hepatorenal injury in ducklings fed peanut meal or Aspergillus flavus extract.* Path. Vet. 1:105.

Nyfeldt, A.: 1934. Étude sur les leucoses des poules. I. Une myéloblastose pure. Sang 8:566.

Oberling, C., and Guérin, M.: 1934a. La leucémie érythroblastique ou érythroblastose transmissible des poules. Bul. Assoc. franc. p. l'étude du cancer 23:38.

————, and Guérin, M.: 1934b. Formations de moelle osseuse hétérotopique d'aspect tumoral chez la poule. Bul. Assoc. franc. p. l'étude du cancer 23:341.

————, Guérin, M., and Lacour, F.: 1953. Forme leucémique de l'endothéliome de Murray-Begg obtenue sous l'influence des rayons X. Bul. Cancer 40.170.

Olson, C., Jr.: 1936. A study of transmissible fowl leukosis. Jour. Am. Vet. Med. Assoc. 89:681.

————: 1940. Transmissible fowl leukosis. A review of the literature. Mass. Agr. Exper. Sta. Bul. 370.

————: 1941. A transmissible lymphoid tumor of the chicken. Cancer Res. 1:384.

————: 1942. A study of neoplastic diseases in a flock of chickens. Am. Jour. Vet. Res. 3:111.

————: 1945. *The immunizing action of a lymphoid tumor in chickens.* Am. Jour. Vet. Res. 6:103.

————: 1947. Immunization against a lymphoid tumor of the chicken. IV. Use of miscellaneous tissues. Cornell Vet. 37:251.

————: 1948. Spontaneous lymphocytoma in a flock of chickens. Am. Jour. Vet. Res. 9:198.

————, and Bullis, K. L.: 1942. A survey and study of spontaneous neoplastic diseases in chickens. Mass. Agr. Exper. Sta., Bul. 391.

————, and Dukes, H. H.: 1933. The basal metabolic rate of chickens affected with fowl paralysis, transmissible fowl leukosis and certain spontaneous neoplasms. Folia Haemat. 60:57.

————, and Rountree, J.: 1957. Unpublished data.

Ontario Department of Agriculture, 1945: Report of the Ontario Veterinary College Sessional, Paper No. 29, 1945.

Oshima, F., and Roki, K.: 1925. Studien uber die Hühnergeschwulste. Trans. Jap. Path. Soc. 15,279.

Peacock, A., and Peacock, P. R.: 1956. Methylcholanthrene-induced tumours of glandular epithelium in fowls. Brit. Jour. Cancer 10.110.

Peacock, P. R.: 1946. The etiology of fowl tumors. Cancer Res. 6.311–28.

Pentimalli, F.: 1915–1916. Ueber die Geschwulste bei Huhnern. I. Mitteilung. Allgemeine Morphologie der spontanen und der transplantablen Huhnergeschwülste. Zeitschr. f. Krebsforsch. 15:111.

————: 1941. Transplantable lymphosarcoma of the chicken. Cancer Res. 1:69

Perek, M.: 1960. An epizootic of histiocytic sarcomas in chickens induced by a cell-free agent. Avian Dis. 4:85.

Petit, G., and Germain, R.: Quoted by Pohl.

Peyron, A., and Blier, J.: 1927. Sur un nouveau cas de tumeur transplantable chez les oiseaux. Myome malin chez un coq. Bul. de l'Assoc. franc. pour l'étude du cancer 16:516.

Pohl, R.: 1926. Beitrage zur Pathologie der beim Haushuhne auftreienden Geschwulste. Arch. f. wiss. u. prakt. Tierheilk. 54:142.

Prospero: Quoted by Heim.

Reinhardt, R.: 1930. Die pathologisch-anatomischen Veränderungen bei den spontanen Krankheiten der Hausvögel. Ergebn. der allg. Path. u. path. Anat. 23:553.

Reis, J., and Nóbrega, P.: 1955a. Tratado de doenças das aves. Inst. Biol. São Paulo, Brazil, Vol. 1, pp. 219–569.

————, and Nóbrega, P.: 1955b. Doenças de aves em São Paulo. Análise de 17,753 cases. Arq. do Inst. Biol. 22:119.

Reitsma: Quoted by Hoogland.

Rigdon, R. H.: 1954. Spontaneous occurrence and regression of hemangiomas in chickens. Southwest. Vet. 7:311.

————, and Brashear, D.: 1954. Experimental production of squamous-cell carcinoma in the skin of chickens. Cancer Res. 14:629.

Rubin, H.: 1960. A virus which induces resistance *in vitro* to infection with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S. 46:1105.

————, Fanshier, L., Cornelius, A., and Hughes, W. F.: 1962. Tolerance and immunity in chickens after congenital and contact infection with an avian leukosis virus. Virology 17:143.

————, and Vogt, P. K.: 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. Virology 17:184.

Savage, A.: 1926. Adenocarcinoma of gallbladder in a hen. Cornell Vet. 16.67.

Schlumberger, H. G.: 1954. Neoplasia in the parakeet. I. Spontaneous chromophobe pituitary tumors. Cancer Res. 14:237.

Schneider, M.: 1926. On the frequency of spontaneous tumors in the domestic fowl. Jour. Exper. Med. 43:433.

Schöppler, H.: 1913. Carcinoma ventriculi cylindrocellulare beim Haushuhn. Zeitschr. f. Krebsforsch. 13.332.

Seifried, O.: 1923. Das "Oophoroma folliculare" beim Huhn. Ein Beitrag zur Histogenese der epithelialen Ovarialtumoren. Zeitschr. f. Krebsforsch. 20:188.

Sevoian, M., Chamberlain, D. M., and Counter, F.: 1962. Avian lymphomatosis. Experimental reproduction of the neural and visceral forms. Vet. Med. 57:500.

Sheather, A. L.: 1911. Teratoma in a cock. Jour. Comp. Path. and Therap. 24:129.

Simpson, C. F., Anthony, D. W., and Young, F.: 1957. Visceral lymphomatosis in a flock of turkeys. Jour. Am. Vet. Med. Assn. 130.93.

Teutschlaender, O.: Quoted by Heim.

Waters, N. F.: 1945. Natural transmission of avian lymphomatosis. Poultry Sci. 24:226.

————: 1947. The contagious nature of a lymphoid tumor in chickens. Science 106 246.

————. 1954. Etiological relationship of visceral and neural lymphomatosis. Poultry Sci. 33:365.

Wickware, A. B.: 1946. The incidence of erythroleucosis following inoculation by various routes. Canad. Jour. Comp. Med. 10:74.

Wight, P. A. L.: 1962. Variations in peripheral nerve histopathology in fowl paralysis. Jour. Comp. Path. 72:40.

Zannini: Quoted by Heim.

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33

External Parasites of Poultry

Ectoparasites of poultry comprise a relatively large group. Certain species are well known, but it is difficult to evaluate the importance of some because their distribution has not as yet been accurately determined. Furthermore, those parasites believed today to be of relatively minor importance may prove later to need more attention as their number increases or as they become more widely distributed or recorded. Because of the large number of species of external parasites of bird hosts, this discussion will primarily be limited to those of the domesticated chicken, turkey, guinea fowl, duck, goose, and pigeon of the mainland of North America.

Before considering the external parasites individually, it appears advisable to review the group. This will orient them with relation to the internal parasites and to the animal kingdom in general.

Basically, external parasites are all those living forms which, for the purpose of

securing food, live on the exterior of the host's body. Thus might be included not only animal parasitic forms but also certain viruses, and parasites belonging to the plant kingdom, such as the bacteria, molds, fungi, and yeasts, some of which attack the skin or feathers. However, of chief concern are those *animal* forms that live as parasites on birds. Even some of these do not confine themselves entirely to the surface, although probably at some remote period of evolution they were strictly external parasites. Examples of this peculiarity include the scaly-leg mite that tunnels into the epithelium of the lower legs, certain quill mites that enter the quill bases, and the subcutaneous and airsac mites that live beneath the skin and in the internal organs, respectively.

Certain ectoparasites of birds actually eat the dead cells of the skin and its appendages, e.g., lice. However, for most of them the skin merely serves as a conven-

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ient medium through which they draw blood or lymph and from which they obtain warmth and shelter.

Ectoparasites may be closely confined to their hosts during the entire life cycle, as is true for bird lice, transmission taking place by host contacts. Others wander freely from bird to bird. Some are highly host specific, which contradicts the viewpoint that chicken lice, for example, propagate on horses or other animals. On the other hand, some species may maintain a rather loose relationship to their food supply. Adapted as they are to living on birds, they do not always confine their activities to one particular host species or even to birds as a group. Such forms include certain of the host-cosmopolitan insects: the gnats, mosquitoes, bedbugs, and fleas. Other external parasites, e.g., the fowl tick and the common red mite, attack birds only at night, hiding in surrounding shelters during the daytime.

Variations in habits such as noted above are important when control measures are to be considered. Mites, as a group, cannot be successfully controlled by any single method of attack because of habit variations among species. This indicates the necessity for accurate identifications as a preliminary. In case of doubt the various state and national diagnostic services may be called upon for assistance.

CLASSIFICATION

Practically all external parasites of birds belong in the invertebrate animal group (phylum ARTHROPODA). The arthropods are jointed-limbed animals without a vertebral column. Nearly all those parasitic on birds and on other animals are further characterized by having tracheal tubes for breathing.

Arthropods with antennae include the parasitic insects (class INSECTA), such as lice, "bedbugs," fleas, beetles, flies, and gnats. Of these, only the last three have wings. The insect body is divided into head, thorax, and abdomen. The legs and the wings (if present) are attached to the thorax. Insects are further distinguished by having three pairs of legs in the adult stage.

Arthropods not having antennae include the spider-related arachnids (class ARACHNIDA) of which many of the mites and all of the ticks are parasites. The arachnid body consists of a combined head and thorax (cephalothorax) not usually marked off from the unsegmented abdomen. The legs of arachnids are attached to the cephalothorax. There are typically four pairs of legs in the adult and the nymphal stages, whereas the larval arachnid is provided with only three pairs.

CONTROL IN GENERAL

The use of insecticides and acaricides on domesticated poultry may be divided into two eras: prior to 1940 and after 1940.

Prior to 1940 the following chemicals and methods had been used for many years: Dust baths containing wood ashes, "road" dust, tobacco, slaked lime, and sulfur; liquids, powders and ointments containing sulfur, caraway oil, balsam of Peru, mercury compounds, pyrethrum, derris, sodium fluoride, sodium fluosilicate, naphthalene; fumigants such as sulfur dioxide, hydrogen cyanide; the use of heat by the application of scalding water to buildings and equipment.

Many of the preceding therapies were quite effective and economical but, since 1940, most of them have been abandoned.

Attempts to control external parasites of poultry by means of chemicals administered internally (systemic therapy) via feed and water apparently originated on a commercial scale in the early 1920's. Parman and associates (1928) tested 30 such substances but none was of practical value. From 1937 to 1951 other workers attempted systemic therapy without significant success (Emmel, 1937; Creighton et al., 1943; Richter and Insko, 1948a; and Menon et al., 1951). More recently there is renewed interest in systemic therapy. The results are somewhat encouraging as described by Kraemer and Furman (1959),

Hoffman (1961), and Furman and Pieper (1962) and others.

After 1940 and especially since 1945 (the end of World War II) an impressive number of organic chemicals have been manufactured for the control of ectoparasites. Beginning with DDT the list has grown rapidly and is still increasing almost monthly. The poultry producer and feeder are confronted with a rather confusing array of products and claims for efficiency. Limitations of space in this chapter make it impossible to discuss each of the newer pesticides; their formulation, dosage, methods of application, efficacy, toxicity, and the contraindications to their use. Instead, it might be advisable to first summarize the more important criteria for an acceptable insecticide or acaricide as follows:

1. It should kill one or more species or groups of parasitic insects, mites, or ticks.

2. It should be economical to the user and profitable to the manufacturer and dealer.

3. It should be in an easily prepared form and it must maintain its potency for a reasonable length of time.

4. Directions for its dosage and application should be plainly and completely stated. The user must be informed whether it is to be applied to individual birds or to flocks directly; or to litter, nests, roosts, dropboards, walls, soil, or to the air of the poultry building. Mechanical devices such as sprayers and powder blowers should be specified. The exact method of dilution, if any, should be stated.

5. Any possible toxicity to birds should be clearly indicated; especially the effects on production and fertility (Smyth, 1956; Albert, 1962).

6. The user should be warned regarding toxicity of pesticides to man during their application, or later as the result of chemical accumulations (residues) in and about poultry buildings and on clothing.

7. Obviously, a chemical must not be toxic to the consumers of poultry meat and eggs (Smyth, 1956) nor should its use have

any detectable effect upon the odor or flavor of poultry products.

Research may eventually bring about the development of efficient and safe insecticides and acaricides. Until then the factor of human error must constantly be considered. The newer chemicals should be field-tested until their value in all respects is proved. Perhaps the present-day pesticides may eventually be replaced by nonchemical biological control methods (see Rietz, 1960; deOng, 1960) including preventive and therapeutic immunization of poultry against external parasites.

SPECIFIC RECOMMENDATIONS FOR CONTROL

Several recent publications may be used as guides to the development and use of pesticides (deOng, 1956; Metcalf, 1957; Frear, 1961, 1962).

Specific control measures may be obtained from manufacturers and their representatives, from veterinary parasitology books and related periodicals as well as from the *Journal of Economic Entomology* and from *Poultry Science*. The bulletin offices of state agricultural experiment stations and the office of information of the United States Department of Agriculture may be appealed to for publications on external parasites of poultry. Local veterinary practitioners, extension veterinarians and entomologists, also veterinary diagnostic laboratories are excellent sources of information and service.

Annually the entomology staff of the Iowa State University of Science and Technology prepares a "Summary of Iowa Insect Pest Control Recommendations" under the sponsorship of the Cooperative Extension Service (Publication IC-328). The following information is quoted from that publication which was issued January 1961:

I. Against chicken lice, bedbugs, and roost mites:

CO RAL: Add 6 ounces of 25 per cent Co Ral wettable powder to 15 gallons of water for spraying 500 to 600 chickens. Spray roosts and nests also. In dust boxes, use 1 ounce

of 25 per cent Co-Ral wettable powder to 3 pounds of sand, dust, or talc for 50 birds. Do not use within 7 days of slaughter.

MALATHION: Use 1 pint of 50 to 57 per cent malathion emulsifiable concentrate in 6½ gallons of water (enough for 6,500 square feet of surface). Spray walls, ceilings, roosts, nests, and litter. Spray birds lightly.

OR: Use 1 pound of 4 or 5 per cent malathion dust scattered on 50 to 60 square feet of litter. Put a handful in each nest. The spray method does a faster job of control.

SEVIN: Use 2 pounds of 50 per cent Sevin wettable powder in 25 gallons of water Spray 1 to 2 gallons for 1,000 square feet of wall, bedding, litter, and roost surfaces Do not treat litter in nests

OR: Use 1 pound of 5 per cent Sevin dust per 100 birds. Apply by means of a shaker can or hand duster Apply 1 pound of dust to each 40 square feet of litter and roosts. Do not treat litter in nests.

2. Against the northern fowl mite or feather mite:

CO-RAL Use dust boxes as recommended against lice, bedbugs, and roost mites

MALATHION: Use 1 pint of 50 to 57 per cent emulsifiable concentrate in 6½ gallons of water. Spray the vent area of each chicken lightly, as well as the entire house. Also use a dusting box containing 1 pound of 4 or 5 per cent malathion dust in 3 pounds of talc, flour, or wood ashes.

SEVIN: Use as recommended against lice.

3 Against lice on turkeys on range:

CO RAL: Apply 15 ounces of 25 per cent Co-Ral wettable powder in 25 gallons of water per 2,500 to 3,000 turkeys. Do not use within 7 days of slaughter.

MALATHION: Use 2 quarts of 50 to 57 per cent emulsifiable concentrate in 25 gallons of water to treat 5,000 turkeys Use a crop-sprayer boom on the ground. Spray upward with a fine spray and drive the birds over the boom. Do not use within 7 days of slaughter.

SEVIN Use as for lice on chickens.

Continuing to quote from the Iowa State University, Cooperative Extension Service, Publication IC-328 (January 1964):

Urge insecticide users to read and follow di rections on the label. Avoid insecticide use when wind currents are such that the chemical might drift onto adjacent forage crops or pastures. Failure to take all precautions may result in excessive residues appearing in meat, milk, or harvested crops. Such contamination may result in seizure by governmental agencies. ALL INSECTICIDES ARE POISONS AND SHOULD BE USED WITH CARE. Do not overdose. Wash hands following use of chemicals, and if any is spilled on the clothing, change to clean, dry garments. Completely destroy all empty insecticide containers. Do not reuse. Many of the organic phosphorus insecticides break down rapidly in alkaline water (pH above 7.0). We find that the pH of municipal water supplies in all of Iowa's larger cities and towns ranges from 8.0 to 9.0. This is alkaline enough to reduce the killing power of the insecticide in a short time. To counteract alkalinity, add 1 to 2 teaspoonfuls of vinegar per gallon of water. Unsoftened well water ranges from pH 7.2 to 7.5. The addition of one half teaspoonful of vinegar per gallon will make such water slightly acid. You can determine the pH of your water supply by using a soil testing kit; or consult your city water department.

LICE

These insects are the most common and widespread external parasites of birds. They all belong in the order MALLO-PHAGA, these lice having chewing mouthparts. Most authorities agree that there are more than 40 species of lice reported from domesticated fowls. A survey of the literature, however, shows considerable variance in the listing of North American species and a decided disagreement on the scientific names assigned to them. Fortunately, as far as the veterinarian and the poultry raiser are concerned, the various species of bird lice are at present all controlled by the same methods. Birds frequently harbor several species of lice at the same time.

The following list of lice of North American poultry is from Hopkins and Clay (1952) and from Emerson (1956).

Chicken lice:

Cuclotogaster heterographus, head louse
Goniocotes gallinae, fluff louse
Goniodes dissimilis, brown louse
Goniodes gigas, large body louse

Lipeurus caponis, wing louse
Menacanthus cornutus, body louse
Menacanthus pallidulus, small body louse
Menacanthus stramineus, body louse
Menopon gallinae, shaft louse

Turkey lice:
Chelopistes meleagridis, large body louse
Menacanthus stramineus, body louse
Oxylipeurus polytrapezius, slender louse

Guinea fowl lice:
Goniocotes gallinae, fluff louse
Goniodes dissimilis, brown louse
Goniodes gigas, large body louse
Goniodes numidae, feather louse
Menacanthus stramineus, body louse
Menopon gallinae, shaft louse

Duck and goose lice:
Anaticola anseris, slender louse
Anaticola crassicornis, slender louse
Anatoecus dentatus, duck and goose louse
Menopon gallinae, shaft louse
Menacanthus stramineus (rarely), body louse

Pigeon lice:
Campanulotes bidentatus, small louse
Coloceras damnicorne, little feather louse
Colpocephalum turbinatum, narrow body louse
Columbicola columbae, slender louse
Hohorstiella lata, large body louse

Lousiness (pediculosis) of birds is diagnosed by finding on the birds wingless, dorsoventrally flattened, brownish-yellow, quickly moving insects. In size, bird lice vary from somewhat less than 1 mm. in length to the largest species which are slightly more than 6 mm. long (Figs. 33.1, 33.2, 33.3, 33.4, and 33.5). See also line drawings in Whitehead (1942) and Emerson (1956).

Lice spend the entire life cycle on the host. Eggs are attached, often in clusters, to the feathers. The entire life cycle takes about 2 to 3 weeks for completion. One pair of lice may produce 120,000 descendants within a period of a few months. Their normal life span is several months, but away from the birds they can remain alive only 5 or 6 days.

Although bird lice ordinarily eat cast-off bits of skin and feathers and fragments of feces that adhere around the vent, it has been shown by Wilson (1933) that *Menacanthus stramineus*, the body louse of chickens, may puncture soft quills near the bases and consume the blood that oozes out. This was confirmed by Crutchfield and Hixson (1943), and, in addition, they stated that the body louse draws blood by gnawing through the covering layers of the skin itself.

Severe lousiness in poultry originally was thought to follow malnutrition and lead to weight loss as well as to low production. There is conflicting evidence on these hypotheses. Warren et al. (1948) found no effect on laying records even following rather heavy louse infestation. In 1961, Tower and Floyd (1961b) studied the effect of the body louse on egg production in New Hampshire pullets; they found no significant differences between infested and noninfested birds. Kartman (1949) concluded that lousiness is not necessarily an expression of malnutrition of the host; indeed the contrary appeared to be true.

He also noted that debeaking the birds increased the number of lice present. Edgar and King (1950), studying the effect of moderate infestation by the common body louse, concluded that louse-free hens averaged about 11 per cent greater egg production than did those infested, a difference in net income of 75 to 85 cents per bird. Differences in body weight and in mortality between the two groups were not significant. A more recent study by Gless

and Raun (1959) revealed that an average of 23,000 body lice per chicken reduced egg production from 15 to 84 per cent during a 14-week period.

It might be concluded that lice are not highly pathogenic to mature birds. However, there appears to be clinical evidence that lice irritate nerve endings, thus interfering with the rest and sleep so necessary to immature animals. Louse-infested chicks may die. Also, lousiness frequently
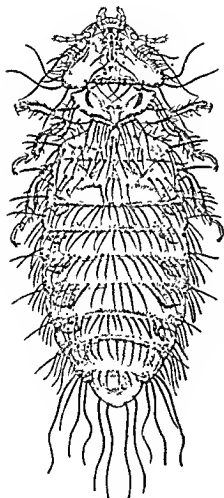
FIG. 33.1 — Menopon sp. Chicken louse, male. ×53. (Reis and Nobrega.)
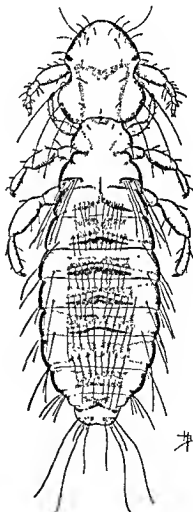
FIG. 33.2 — Cuclotogaster heterographus. Head louse of chickens, male. Greatly enlarged. (U.S.D.A., Bur. Entom. and Plant Quaran.)
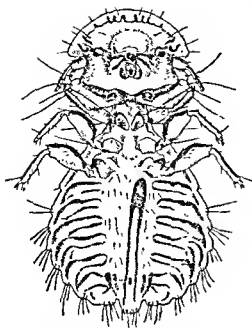
FIG. 33.3 — Goniocotes sp. Chicken louse, male. X20. (Reis and Nobrega.)

accompanies manifestations of poor husbandry such as internal parasitism, infectious diseases, malnutrition, and insanitation.

Howitt et al. (1918) isolated the virus of equine encephalomyelitis from the body louse (Menacanthus stramineus). More recently, Meyer and Eddie (1960), also Eddie, Meyer, Lambrecht, and Furman (1962) isolated the virus of ornithosis from Menopon gallinae, the shaft louse, and from mites on chickens and turkeys.

Control of lice. This problem primarily involves the birds themselves, although in heavy infestations the houses should be cleaned and disinfected. Lice multiply most rapidly during cold weather when birds are in closer contact; therefore, it is essential to combat them during the mild days of the fall season so that birds may go into production in the best of health. Once a flock is free from lice, it is important to isolate new additions until their freedom from these parasites has been assured. The use of insecticides against poultry lice is discussed on page 926 under Control in General.

"BEDBUGS" AND ALLIED INSECTS

The insect order HEMIPTERA includes the true bugs, several species of which parasitize birds by sucking blood. Two families are of particular interest. The family Cimicidae contains the true "bedbugs," and the family Reduviidae includes the "assassin bugs." The latter ordinarily are predaceous on other insects; however, some species do attack man and animals.

The true "bedbugs" are flattened dorsoventrally, thus allowing them to creep into crevices where their young are raised. The adults measure about 2 to 5 mm. in length by 1.5 to 3 mm. in width. The color varies according to species from brown to yellow or red. They have small padlike wing remnants. The suctorial mouthpart structure or "beak," which is jointed, folds under the head and part of the thorax when not in use. Deeply pigmented eyes are prominent on the head. The abdomen has eight segments. Stink glands provide the common "bedbug" and its surroundings with an unpleasant odor.

The female "bedbug" lays several 1 mm. sized eggs per day in crevices until about 200 have been deposited. The eggs hatch in about 10 days. There are five nymphal stages, the nymphs feeding at each stage and hiding to digest the meal of blood and to molt their skins. From egg hatching to adulthood requires about 40 days. Nymphs may withstand starvation for about 70 days, and the adults may live about 1 year without food. Feeding usually occurs at night, the bugs becoming engorged with blood within 10 minutes.

If attacked by large numbers of bugs, young birds especially may be seriously depleted of blood. The bites are usually followed by swelling and itching due to the injection of saliva into the wound.

The most widespread of the "bedbugs" is Cimex lectularius (Fig. 33.6), which attacks man and most mammals and poultry. It is most prevalent in temperate and subtropical climates. Poultry houses

FIG. 33.4 — Goniodes sp. Chicken louse, male. ×42. (Reis and Nobrega.)
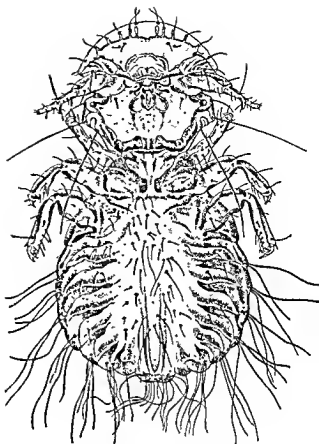
and pigeon lofts may become heavily invaded.

Other "bedbugs" reported to attack birds include the following species:

Haematosiphon inodora, the Mexican chicken bug, adobe bug, or "coruco," which also occurs in southern and western United States and in Central America. Usinger (1947) has found it in the nests of the California condor and of the great horned owl in Oklahoma Lee (1955a) gives an account of its biology and mentions the turkey as a new host in New Mexico and Arizona. It also attacks man.

Oeciacus vicarius, commonly found in the nests of swallows (particularly barn swallows) whence they may spread to poultry and to man (Myers, 1928).

In addition to the above, numerous other species of the true "bedbug" family have been reported on birds in various countries outside the United States. The insect order HEMIPTERA also includes bugs of the family Reduviidae as previously mentioned. These are usually of minor interest in avian parasitism. "Assassin bugs," "cone-nosed bugs," and reduviids are terms applied to such insects, of which there are many species. Only a few of them have learned to suck the blood of mammals and birds. They are larger than true "bedbugs," being up to 25 mm. in length, and they have well-developed wings; otherwise their morphology, life cycles, and behavior are quite similar. Species of reduviid bugs reported as attacking poultry in the United States include *Triatoma sanguisuga* in Maryland, Florida, California, and Texas, and *Triatoma protracta* in Utah and California. It is of interest to note that in 1910, *Triatoma sanguisuga* was found to harbor the virus of equine encephalomyelitis in
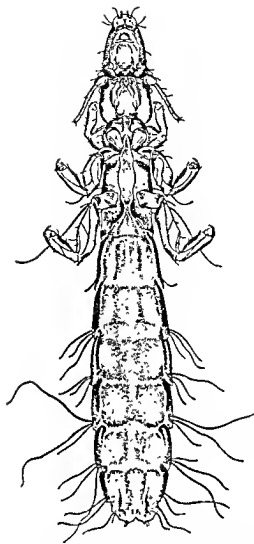
FIG. 33.5 — Columbicola columbae. Slender pigeon louse, female. ×48. (Reis and Nobrega.)

FIG. 33.6 — Cimex lectularius. Common bedbug, male. ×15. (Benbrook and Sloss.)

Kansas by Kitselman and Grundmann (1910). This virus has also been found in natural infections in pigeons and pheasants.

Control of "bedbugs" and allied insects. Treatment must be directed at the birds' surroundings during the daytime, because the bugs ordinarily feed at night. In the past, fumigation with hydrocyanic acid gas or with the sulfur dioxide released by burning sulfur was considered to be an effective control method. The use of in-secticides against "bedbugs" is discussed on page 926 under Control in General.

FLEAS

Many species of fleas have been found on birds, but only six species have been reported from poultry in this country. However, fleas are very adaptable blood-sucking insects and may attack various host species. They are cosmopolitan in distribution although more abundant in temperate and warm climates.

They may be recognized as brown to black, laterally flattened insects having the ability to run rapidly along the skin and to propel themselves in the open by leaping. In size they vary from 1.5 to 4 mm. in the adult stage. The adults suck blood one or more times during the day, although some species are nocturnal. The sticktight flea usually remains attached to the host for days or weeks at a time. Female fleas deposit several eggs per day which roll off the host into surrounding litter where they incubate. Dampness is

essential for further development. The eggs are white, almost spherical bodies less than 1 mm. in diameter. Within one to several weeks, depending upon species and climate, the eggs hatch, liberating tiny maggotlike larvae that feed partly on organic matter found in dust and litter, but their principal food is flea feces deposited conveniently by the adult fleas. This flea feces is rich in host bloodproducts, thus providing a highly nutritious diet for the larvae. After the larvae have grown and shed their skins, usually twice in a period varying from one to several weeks, they proceed to spin silken cocoons, entangling the thread with various particles of dust and dirt. Then follows the inactive pupal stage for a period varying from one week to months, depending upon the temperature. During this period the pupae transform into white, then yellow, then brown fleas. Emerging from the pupal cocoons, the young fleas seek a host, suck blood, and reach maturity within a few days.

Immature fleas may live for weeks or months without food. Adult fleas may also live for weeks without feeding, but when a host is available their life span may extend over many months to a year or more. The total length of the life cycle is thus seen to vary greatly depending upon such factors as temperature, humidity, exposure, and host availability.

The fleas of domestic poultry in North America include the following species: *Echidnophaga gallinacea*, the sticktight flea; *Ceratophyllus gallinae*, the European chicken flea; *Ceratophyllus niger*, the western chicken flea; *Ctenocephalides felis*, a cat flea; *Pulex irritans*, the human flea; and *Orchopeas howardii*, a squirrel flea. These will be discussed before flea control is considered.

Echidnophaga gallinacea (Fig. 33.7), the cosmopolitan sticktight or tropical chicken flea, more often occurs in the southern United States, although occasionally it is found as far north as New York. The adult is about 1.5 mm. long and is reddish-brown in color. These fleas usually
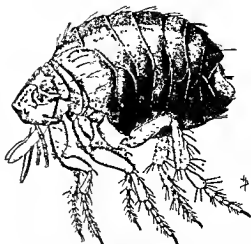
FIG. 33.7 — *Echidnophaga gallinacea*. The sticktight or tropical chicken flea, female. Greatly enlarged. (U.S.D.A., Bur. Entom. and Plant Quaran.)

attach to the skin of the head, often in clusters of a hundred or more. The mouthparts are deeply embedded into the skin so that it is difficult to dislodge them. The adult females forcibly eject their eggs, so that they reach surrounding litter, one to four eggs per day being produced. Incubation takes from 4 to 14 days; the larval period lasts from 14 to 31 days; the pupal period for 9 to 19 days; and the newly emerged fleas mature from 11 to 18 days.

The sticktight flea has been reported from the following hosts: chicken, turkey, pigeon, blackbird, bluejay, hawk, owl, pheasant, quail, sparrow; also man, horse, cattle, swine, dog, fox, cat, badger, coyote, deer, ground squirrel, lynx, mouse, opossum, rabbit, raccoon, rat, ring-tailed cat, and skunk.

This flea has not been accused of carrying infectious disease agents to chickens. The irritation and blood loss attributed to it may damage poultry seriously, especially young birds, in which death may occur. Production is lowered in older birds. Alicata (1942) experimentally transferred the rickettsia of human endemic (murine) typhus from infected rats to guinea pigs through the agency of sticktight fleas, thus indicating a possible public health importance of this parasite.

Ceratophyllus gallinae (Fig. 33.8), the European chicken flea, also occurs in the United States. It has been reported from Maine, Massachusetts, Connecticut, New York, Delaware, Michigan, and Iowa. Undoubtedly, it has a much wider distribution. The hosts include the chicken, pigeon, bluebird, sparrow, and tree swallow; also man, dog, chipmunk, rat, and squirrel. The adult female measures from 3 to 3.5 mm. in length. This flea behaves like most fleas in that it stays on birds only long enough to feed, its breeding activities occurring in the nests and other surroundings. Otherwise, its effects are like those of the sticktight flea.

Ceratophyllus niger, the western chicken flea or black hen flea is reported mainly from the Pacific Coast area, although Bigland (1955) found it in Alberta, Canada. It may attack various mammals and birds including the chicken, turkey, cormorant, gull, magpie, sparrow, and woodpecker; also man, mouse, and rat. Its principal breeding place is in birds' nests. Grossly and in habit it resembles *C. gallinae*.

Ctenocephalides felis is known as the cat flea. It has also been reported from the chicken, pheasant, dog, fox, man, bobcat, coyote, opossum, rabbit, raccoon, rat, shrew, squirrel, and woodchuck. This flea is only an incidental pest of poultry in North America. The adult female may be 3 mm. in length.

Pulex irritans is a flea primarily of man but it may attack chickens. Other hosts are swine, dog, fox, cat, badger, coyote,

deer, ground squirrel, guinea pig, lynx, mountain lion, opossum, prairie dog, rabbit, raccoon, rat, skunk, squirrel, weasel, wild swine, and burrowing owl. The adult female may be 4 mm. long.

Orchopeas howardii, a flea ordinarily found on squirrels, has been noted to attack chickens in Massachusetts, according to Shaw and Clark (1953). Other hosts include the dog, fox, cat, man, bobcat, chipmunk, coyote, mink, mole, mouse, opossum, rabbit, raccoon, rat, shrew, squirrel, weasel, woodchuck, owl, and swallow. The adult female is about 2.5 mm. in length.

Fox (1940) and Hubbard (1947) report many other species of fleas found on birds other than domesticated poultry.

Control of fleas. This involves not only ridding the birds themselves of fleas, particularly the sticktight flea, but also ridding the nests and housing areas of these parasites. In this respect, flea control methods are quite similar to those used against red mites and bedbugs.

Many homemade and commercial therapeutic agents have been recommended against fleas as pests of man and animals, for example, see Eads (1946), Roberts *et al.* (1947), Stage (1946), Smith (1951), U.S. Department of Agriculture (1955), and Iowa State University (1964). Control of the sticktight flea, *Echidnophaga gallinacea* was reported by Rodriguez and Riehl (1961) using 4 per cent malathion dust. This was applied twice at 28-day intervals and effective control lasted for 150 days after the second application. The mala-

thion dust was placed in dusting boxes or in soil wallows at the rate of 15 pounds per 100 chickens. In addition, the floors were covered with the 4 per cent malathion dust at the rate of 1 pound per 20 square feet.

Poultry, dogs, cats, and rats should be screened away from under buildings, as they may serve to perpetuate flea invasions. Sunlight, hot dry weather, excessive moisture, and freezing hinder the development of fleas; whereas darkness, coolness, dampness, and warmth favor them.

ADULT BEETLES AND BEETLE LARVAE

Beetles are included in the insect order COLEOPTERA. They are stoutly armored insects, having two pairs of wings, the membranous hind wings being covered by horny, sheathlike fore wings termed elytra. The adult female beetle lays eggs from which hatch larvae, commonly called grubs, followed by a pupal stage. The adults and larvae show great variation in habits. Some live on land, others in water. Certain species feed on animal matter, others on plants; thus many species are useful as scavengers or in reducing other insect populations. Numerous species cause considerable harm by destroying plants and plant products or by acting as pests to animals. Other species serve as intermediate hosts for internal parasites.

As far as birds are concerned, certain adult beetles and their larvae may act as pests or may serve as intermediate hosts for internal parasites, particularly certain of the tapeworms. Numerous species of beetles are known to transmit the following tapeworms of poultry: *Raillietina cesticilus*, *Choanotaenia infundibulum*, *Hymenolepis carioca*, *Hymenolepis cantaniana*, and *Raillietina magninumida*. Because beetles make up part of the diet of birds and because some beetles eat avian carcasses, they may transmit bacterial or virus infections; but of this little is known. Theodoridès (1949) lists past records on the beetles injurious to domestic birds and mammals.

Beetle larvae acting as pests of poultry include the following species:

Tenebrio molitor, the yellow mealworm, is ordinarily found in the adult and grub stages consuming grain products stored in mills, warehouses, bakeries, and groceries. The beetles are shiny brown to almost black in color and about 15 mm. long. They may infest setting hens, attacking mainly the feet where the loss of skin may be followed by severe hemorrhage. The larvae or grubs, known as flour, meal, or bran "worms," are smooth, hard, yellow, cylindrical, wormlike creatures about 30 mm. long. These grubs have been found to erode the skin of young pigeons. According to Levi (1957), other related mealworm beetle larvae may produce similar damage.

Alphitobius diaperinus, the lesser mealworm, usually lives on stored grain products. Harding and Bissell (1958) found a heavy infestation of these beetles and their larvae in ground corncobs used as litter for baby chicks. Healthy chicks were not molested but the mealworm larvae had bored into the tissues of those that were dying. This damage might be mistaken for an attack on the living chicks.

Dermestes lardarius, the larder beetle, and related species, ordinarily destroy stored grain products and meats (especially ham and bacon) or feed on hides, skins, furs, museum specimens, or decaying animal matter, notably the accumulated droppings in pigeon lofts. The adult larder beetle is about 7 mm. long, black in color, and the basal half of each wing cover is brownish-yellow crossed by a band of three black spots. The larvae are about 12 mm. long, dark brown above, gray below, and are covered with brown hairs. The larvae may attack the skin of nestling pigeons.

Silpha thoracica, *Silpha apaca*, *Necrophorus vestigator*, and possibly other species of the beetle family, Silphidae (carrion beetles), may also breed in pigeon droppings. The larvae, which are about 15 mm. long, and black, are reported to invade the skin of squabs, and the wounds produced may be secondarily infested by fly maggots.

Control of beetle larval invasions. This

is based upon cleanliness of houses or lofts, from which droppings should be removed frequently. Grain and feed storages and hides and skins should be kept away from birds. If infested, such substances should be fumigated or otherwise treated according to directions that may be obtained from the United States Department of Agriculture. Wounds produced by beetle larvae should be gently cleaned, using commercial benzol to destroy the grubs, followed by daily irrigation with a mild disinfectant solution until healing occurs.

Reporting on the control of bedbugs in chicken houses, Kulash and Maxwell (1945) found that a 5 per cent solution of DDT in kerosene also killed adult dark mealworms, *Tenebrio obscurans*. Harding and Bissell (1958) controlled adult and larval lesser mealworms in brooder house litter by spraying with 0.7 per cent emulsifiable concentrate of malathion; or, with 0.5 per cent DDT spray.

Macrodactylus subspinosus, the rose chafer, is a leaf-chewing beetle that, according to Lamson (1922), is highly poisonous when ingested in quantity by chicks, ducklings, goslings, poults, and young game birds. These beetles are found mainly in the regions of the Atlantic Coast, central states, and Middle West. Symptoms of poisoning appear about 4 to 5 hours after ingestion. The birds become sleepy, the wings droop, and muscular weakness develops. Death may occur in 5 to 24 hours. The condition is diagnosed by finding the beetles in the crop. No specific treatment for affected birds is available, although a saline laxative is advisable. Plants infested with rose chafers may be sprayed with lead arsenate solution.

MOSQUITOES

Although mosquitoes are not as important to poultry as they are to man and other mammals, they are of some direct and indirect interest. Some 110 species have been described from North America. How many of these suck blood from poultry is not known.

Mosquitoes are recognized as two-winged insects belonging to the family Culicidae of the order DIPTERA. Most species are about 5 mm. in length, and the wings are characteristically veined and scaled. The legs and the abdomen are long and slender, and the small spherical head of the female is provided with elongated mouthparts for piercing the skin. The male does not suck blood, but does live from plant juices, nectar, and other fluids.

Mosquitoes deposit their eggs on pools of water in which the larval and pupal stages are passed. The adults emerge from the pupal cases, quickly breed, and then seek a host. In warm weather the life cycle is completed in about 7 to 16 days for the more common species. The adults are most active on quiet days, especially toward evening.

That mosquitoes may attack poultry in swarms is stated by Bishopp (1933), who reported the deaths of numerous chickens in Florida. The offending species was *Psorophora confinnis* (syn. *P. columbiae*). Besides blood loss, the birds appeared to show toxicity from the bites. Edgar and Williams (1948) and Edgar et al. (1951) found that over 99 per cent of the mosquitoes in or near chicken houses in Alabama were *Culex quinquefasciatus*, the southern house mosquito. Others belonged in the genera *Culex*, *Anopheles*, and *Aedes*. Mosquito attacks appeared to reduce egg production.

Yates (1953) stated that although the mosquitoes *Culiseta incidens* and *C. inornata* bite man in the Pacific Northwest; they were more important as pests of livestock, including poultry.

Dow et al. (1957) noted a preference for birds, including chickens, as hosts, by *Culex tarsalis*.

MacCreary and Catts (1951) in Delaware, noted loss of blood and restlessness of roosting chickens when poultry houses were invaded by *Aedes sollicitans*, a salt-marsh breeding mosquito. They also remarked on the large numbers of other species of mosquitoes seen in poultry houses, notably *Culex pipiens*, the house mosquito, also *Culex salinarius* and *Anopheles crucians*.

Fowl pox virus is transmitted by the mosquitoes *Aedes stimulans*, *A. aegypti*, and *A. vexans* according to Brody (1936) and also Matheson *et al.* (1931). The first-named species harbored the virus for 2 days, whereas the last named species continued to infect birds up to 39 days after contacting the virus of fowl pox and pigeon pox Mosquitoes also transmit one type of avian malaria (*Plasmodium* sp.) according to Herman (1938a). For the most part the birds affected are the smaller wild species including canaries Davis (1940) has called attention to the relationship of birds and mosquitoes as hosts for the virus of eastern equine encephalomyelitis. Smith *et al.* (1948) infected seven species of mosquitoes with the virus of St Louis encephalitis of man by feeding them on infected chickens. The mosquitoes transferred the virus back to chickens.

Control of mosquitoes. Mosquito control methods, as applied to dwellings and other buildings and their surroundings, may be used to equal advantage in and around poultry houses and poultry-raising areas.

Iowa State University, Cooperative Extension Service (1964) makes the following recommendations for mosquito control:
DDT: Drain seepage or flood pools where possible, or treat all such pools inaccessible to livestock with one-half cup of 2.5 per cent DDT water emulsion per 1,000 square feet of water surface. Treat brush, weeds, and other mosquito resting places with 2.5 to 5 per cent DDT water emulsion every 7 days. Use malathion in gardens and pastures where livestock are grazing.

DDVP: Use 0.75 per cent DDVP water emulsion on stagnant water at the rate of 2 quarts per 1,000 square feet or as 0.75 per cent DDVP oil solution as a fog to control adults. DDVP does not affect birds or wild mammals in the area.

MALATHION: Spray brush, weeds, and low trees around standing water accessible to livestock with 1 per cent malathion water emulsion every 5 to 7 days.

Keep grass and weeds cut around buildings and spray or dust (4 per cent malathion dust) gardens, flower beds, hedges, and shrubs every 5 to 7 days as long as needed.

A concise discussion of mosquitoes and their control may be found in a leaflet from the U.S. Department of Agriculture (1962c).

Preventive measures by screening and the use of repellents are of doubtful practical value in poultry husbandry.

FLIES AND GNATS

The insect order DIPTERA includes not only the mosquitoes but also many families of flies and gnats. Certain species of these groups may cause harm to poultry in several ways: by sucking blood, by injecting toxic substances, by acting as intermediate hosts for certain tapeworms, by harboring botulinus toxins, or by larval invasion of body openings and skin wounds.

The Pigeon Fly

Pseudolynchia canariensis (Fig. 33.9), the pigeon fly, louse-fly, or flat fly is a rather important parasite of domesticated pigeons in warm or tropical areas. It has been known since 1896 in the southern half of the United States and also occurs in many other countries.

The adult fly is dark brown and about 6 mm. in length. The two transparent wings are somewhat longer than the body. These flies move rapidly through the feathers, and they suck blood, particularly from nestling pigeons about 2 to 3 weeks of age. They may also bite man, inflicting a painful skin wound that persists for several days.

The female pigeon fly deposits on the birds her white larvae, about 3 mm. in length, each enclosed in a pupal case. These roll off the host into the nest, and in a few hours the pupal case hardens and turns black. After a pupal stage of about 30 days, the flies emerge, living for about 45 days, during which time the female deposits four or five young.
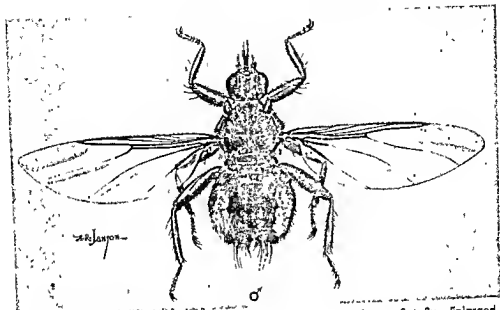
FIG. 33.9 — Pseudolynchia canariensis. The pigeon fly, louse-fly, or flat fly. Enlarged. (Drake and Jones.)

Infested pigeons suffer from blood loss and from irritation. Also, the pigeon fly may transmit a protozoan blood-cell parasite, *Haemoproteus columbae*, the cause of a malarialike disease of pigeons.

There are several other louse-flies related to the pigeon fly that infest various species of wild birds, notable among which is the fly *Lynchia hirsuta*, a transmitter of *Haemoproteus lophortyx*, the cause of California valley quail malaria.

Control of the pigeon fly. Because these flies breed in pigeon nests, it is essential to clean the nests and surroundings at 15- to 20-day intervals and to burn or bury the cleanings. Pigeon lofts may be rid of adult flies, according to Levi (1957), by using a pyrethrum-containing fly spray (1 part pyrethrum extract to 2 parts of kerosene). This should not be sprayed on unhatched eggs. Pigeons may be freed from the flies by applying to them several pinches of fresh pyrethrum or derris powder, rubbing it into the skin. Dipping may be resorted to, in which case derris powder, one-half to 2 ounces, is added to 1 gallon of soft water containing 2½ ounces of laundry soap. Yager and Gleiser (1946)

reported complete kill of pigeon flies on a flock of Signal Corps pigeons by dusting each bird with 3 grams of 10 per cent DDT in talc.

Black Flies

These dipterous insects, variously called turkey gnats and buffalo gnats (Fig. 33.10), belong to the fly family of Simuliidae. They are blood suckers, of which more than 600 species are known (Herms and James, 1961). More than 20 species have been reported to attack domesticated poultry in North America. At times they may cause serious damage to man and to livestock. Their importance to poultry raisers is that they may attack in swarms, depleting the birds' blood volume and injecting toxic material. They also transmit certain blood protozoa belonging to the genus *Leucocytozoon*.

Black flies are tiny, hump-backed, two-winged flies from about 1 to 5 mm. in length and black or nearly black in color. The females are vicious blood suckers during daylight hours. They breed in running or slowly moving water from which they may travel several miles in

FIG. 33.10 — Simulium sp. One of the black flies, buffalo gnats, or simuliids. Enlarged. (Iowa State University)

search of blood. Eggs are laid on solid objects at the edge of water. The larvae emerge in 5 to 30 days and enter the water, attaching to stones or other objects. After about 3 to 10 weeks, during which the larvae molt six times, the pupal stage is reached. This stage, too, occurs under water, lasting from a few days to a month. The adult flies emerge during warm weather. Hibernation occurs in the egg or larval stage.

Simuliids are widely distributed, but they occur mostly from the north temperate to the subarctic regions. Reports of their occurrence in this country date back to the early part of the last century when buffalo gnats seriously interfered with homesteading operations in the South. It was then noted that they would swarm on poultry, forcing setting chickens and turkeys to leave their nests, they killed young birds by forcing their way in large numbers under the wings where they sucked blood.

Walker (1927) reported that Simulium bracteatum fatally attacked goslings in Canada, and Gibson (1930), also of Canada, found that Simulium sp. caused losses to chickens and turkeys. Underhill (1939) stated that Simulium jenningsi (syn. S. nigroparvum) and S. slossonae attacked turkeys in Virginia, as far as 15 miles away from their breeding places. In 1923 heavy losses in chicks occurred in western Iowa. Swarms of gnats produced severe anemia,

leaving a hemorrhage at each skin area punctured. The chicks ingested enormous numbers of the gnats so that their crops were distended with them. Edgar (1953), in Kansas, studied the effects of black fly (Simulium meridionale) bites on egg production in April and May. Production dropped from 70 per cent to 20 per cent in 8 days. One hen died but production became normal shortly after the flies disappeared.

It was not until 1932 that disease transmission by gnats to poultry was proved. Skidmore (1932a), of Nebraska, found that Simulium occidentale could transmit Leucocytozoon smithi, a blood protozoan of turkeys. O'Roke (1934) showed that Simulium venustum transmitted Leucocytozoon simondi to tame and wild ducks in Michigan. Fallis et al. (1956), in Canada, reported the transmission of Leucocytozoon simondi, a blood protozoon of domesticated ducks, by the black flies Simulium croxtoni and S. euradminiculum, also Simulium rugglesi. Anderson (1956), also in Canada, found that six species of black flies transmitted the blood microfilariae of a nematode, Ornithofilaria fallisensis, to domesticated and wild ducks.

Black fly control. This is difficult because these pests breed in streams containing rocks, brush, and logs. Stream clearance may help, but it is an expensive procedure. Sweeping the downstream faces of dams may dislodge many pupae. The drifting smoke of smudge fires will repel the adult flies. Birds may be kept within screened enclosures during the daytime, using screen of 24 mesh per inch or smaller. Repellents are helpful, for example, oil of citronella 1 part in light mineral oil 4 parts may be sprayed on the outside feathers. Dove (1945) quoted Hutson's report on the use of 1 per cent DDT dust for the control of the black fly, Simulium venustum, on golf course greens, tees, and shrubbery in Michigan. A liberal dusting kept the premises practically free of flies for approximately 1 week.

Hocking (1953) attempted chemical

control of the black fly *Simulium venustum* in Manitoba streams. Eight compounds were tested in the laboratory and in streams, including heptachlor, DDT, gamma BHC, and parathion. Results were promising, especially for DDT applications made by helicopter.

Common House Fly

Musca domestica, the common house fly, is frequently eaten by birds. It is an intermediate host for two species of poultry tapeworms, namely: *Raillietina cesticillus* of the chicken, turkey, guinea fowl, and quail; and *Choanotaenia infundibulum* of the chicken and turkey. Skidmore (1932b) reports that common house flies that have fed on infected fowl cholera blood can transmit this disease when fed to turkeys. The common house fly as well as *Lucilia sp.*, a blowfly, were capable of carrying eggs of the cecal worm, *Heterakis gallinae*, which contained the protozoan cause of histomoniasis of turkeys, according to Frank (1953).

Biting Midges

Culicoides spp. are biting midges, "punkies," or "no-see-ums," some 35 species of which have been reported from North America. They attack birds and mammals. Fallis and Wood (1957) report them as intermediate hosts for *Haemoproteus nettionis*, a blood protozoon of domesticated ducks in Canada. MacCreary and Catts (1954) found *Culicoides canithorax* on chickens near salt marshes in Delaware, but they could not determine its relationship to disease.

Stable Fly

Stomoxys calcitrans, the blood-sucking stable fly, attacks most mammals and birds. Fortunately, the bite is not poisonous, although such areas may become infected, and the blood that oozes from bites may attract maggot-producing flies. The stable fly is an intermediate host for *Hymenolepis carioca*, a tapeworm of the chicken, turkey, and quail.

Myiasis

Invasion of birds by fly larvae (maggots) is not as common as in mammals. Knipling and Rainwater (1937) mention that *Cochliomyia hominivorax*, the primary screwworm fly, will deposit eggs in wounds on chickens, turkeys, and geese. Maggots hatching from these eggs actively destroy living tissue. Stewart (1929) reported a case of cloacal invasion of a hen by screwworm larvae. Invaded wounds may be treated with Smear No. 62 or 335, developed in the United States Department of Agriculture and reported by Melvin *et al.* (1941).

The nests of wild birds may become infested by the maggots of various species of flesh and blowflies, with disastrous effects on the nestlings.

Certain fly larvae are of interest because, by breeding on decomposing cadavers, they may ingest toxins of the bacterium *Clostridium botulinum*, according to Bishopp (1923). If poultry eat such maggots, the disease botulism may occur. This has been called "limberneck," a term descriptive of one symptom but not characteristic of botulism alone. The larvae of the following species of flies have been incriminated as transmitters of botulinus toxins, Types A and C: *Lucilia illustris* and *Phaenicia sericata* (blowflies); sarcophagid larvae (flesh flies); and larvae of *Cochliomyia macellaria*, the secondary screwworm fly. Prompt burial, burning, or other sanitary disposal of animal cadavers will do much to prevent botulism from these sources.

Schalk (1928) noted that "fly larvae" developing on tuberculous chicken cadavers could transmit *Mycobacterium tuberculosis* when fed to nontuberculous chickens.

MITES

Numerous and important species of mites may affect domesticated and wild birds. Mites belong in the invertebrate animal group (phylum) ARTHROPODA, in the class ARACHNIDA, and, with the

ticks, in the order ACARINA. For classifications of mites, reference may be made to Baker and Wharton (1952) and Baker et al. (1956).

Parasitic mites are microscopic or barely visible to the unaided eye. The main body parts include a usually unsegmented, soft abdomen broadly continued anteriorly by a combined head and thorax (cephalothorax) to which the legs are attached. Some mites breathe through tracheal tubes, others by absorption of oxygen through the soft skin.

The typical mite life cycle consists of the egg, the larva (six-legged), the nymph (eight-legged but sexually immature), and the adult (eight-legged). The cycle, in general, takes from 1 to 4 weeks for completion, depending upon species, climate, and availability of a suitable host.

Most kinds of birds use blood or lymph for food, hence anemia is a more or less constant symptom. It might be expected that blood-sucking mites could easily transmit bacterial and viral infections. The common nest mite, Dermanyssus gallinae, has been reported by Hertel (1904) and by Plasaj (1925) as a transmitter of fowl cholera organisms; and of the fowl spirochaete, Borrelia anserina, by Hart (1938). The same mite from chickens has been shown by Sulkin (1945) to harbor the virus of equine encephalomyelitis, western type; and by Howitt et al. (1948) to harbor the virus of the eastern type. Reeves et al. (1947) recovered western type equine encephalomyelitis virus from the northern feather mite, Ornithonyssus sylviarum, obtained from the nests of English sparrows and yellow-headed blackbirds.

Hofstad (1949) found that the northern feather mite carried the virus of New castle disease (pneumoencephalitis) of poultry after feeding on infected chickens. Hammon et al. (1948) isolated from the northern feather mite of wild birds a virus or a mixture of viruses from which the St. Louis and western equine encephalomyelitis viruses were obtained.

Smith et al. (1944) isolated human St. Louis encephalitis virus from the red mite, Dermanyssus gallinae, in nature, following which they infected mites from experimental chickens and also infected chickens from mites (Smith et al., 1945, 1946, 1947, 1948). Baker et al. (1956) believe the reported transmission of St. Louis encephalitis virus of man by the chicken mites to be inconclusive. Sulkin and Izumi (1947) recovered the virus of western equine encephalomyelitis from the tropical feather mite, Ornithonyssus bursa. This was confirmed by Miles et al. (1951). On the other hand, three later studies with bird mites from natural sources failed to demonstrate their role as vectors of the viruses of St. Louis encephalitis, or of the eastern and western strains of equine encephalomyelitis virus, according to Chamberlain and Sikes (1955), Reeves et al. (1955), and Sulkin et al. (1955). Meyer and Eddie (1960) isolated the virus of ornithosis (Bedsonia sp.) from feather mites (Ornithonyssus sp.) and also from nonparasitic mites found in the nests of turkeys, two and one-half months after the nests had been abandoned because of ornithosis in the flock.

Those mites that move rapidly over the skin will irritate birds to a considerable degree. Other species burrow into the epithelium, causing tissue proliferation and scab formation. Feather loss results from invasion of feather follicles by certain species, the feather bases being destroyed, or the birds may pull out the affected feathers. Although mites are considered ordinarily to be external parasites, several species invade the subcutis or the internal organs of birds. Red mites and feather mites of birds may bite man, producing papules with itching, according to Boyt (1937), Arnold and Arnold (1943). Mandoul et al. (1945), Berndt (1952), Brown (1955), and Judd (1956).

The rearing of bird mites for experimental purposes has been described by Wisseman and Sulkin (1947) and by Chamberlain and Sikes (1950).
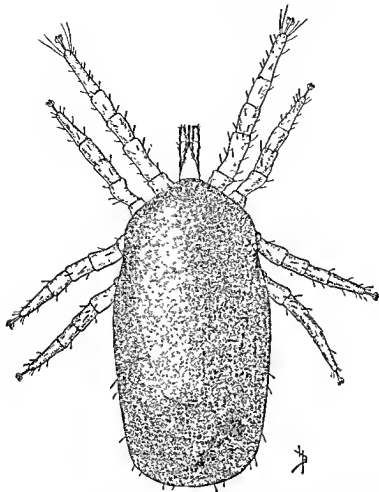
FIG. 33.11 — Dermanyssus gallinae. The red or roost mite, female, after feeding. Greatly enlarged. (U.S.D.A., Bur. Entom. and Plant Quaran.)

Red or Roost Mite

Dermanyssus gallinae (Fig. 33.11), the red or roost mite, is probably the commonest and most widespread of all the mites of birds. Because it breeds in the birds' surroundings, attacking mostly at night, it is apt to be overlooked. The adult female measures about 0.69 by 0.1 mm., varying in color from gray to deep red, depending upon its blood content. Wisseman and Sulkin (1917) have described the life cycle, which may be completed in as little as 7 days. Adult females lay eggs in the hosts' surroundings 12 to 24 hours

after their first blood meal. Eggs hatch in 48 to 72 hours when warm. The six-legged larvae, without feeding, molt in 24 to 18 hours, becoming first stage blood-sucking nymphs; they then molt to second stage nymphs in another 24 to 48 hours, soon afterwards molting to the adult stage. Chickens are the commonest hosts, but turkeys, pigeons, canaries, several wild birds, and man may be attacked. English sparrows frequently transmit this parasite because of the habit of lining their nests with chicken feathers.

Red mites may live for several months without food. When hosts are available

they may not only produce anemia, thereby seriously lowering production, but may actually kill birds through extraction of blood. This is particularly true of young birds and of setting and laying hens. Birds in production may refuse to lay in infested nests. This symptom indicates that poultry houses should be examined for mites.

Control of Red Mites. Among the methods for control were those proposed by Emmel (1942), Alicata *et al.* (1946), Hixon and Muma (1947), Peterson (1949), and Moore and Schwardt (1954). More recent recommendations will be found in references such as Linkfield and Read (1958), Hoffman (1960), Rodriguez and Riehl (1958, 1960b), Hoffman and Drummond (1961), and the U.S. Department of Agriculture (1962b).

In summarizing the more recent methods for red mite control, refer to the Iowa State University Summary of Iowa Pest Control Recommendations on page 927.

To prevent red mite infestation, inspect houses frequently; quarantine new birds until inspected; disinfect old houses before admitting birds, and destroy nearby sparrow nests if possible.

Northern Feather Mite

Ornithonyssus sylviarum (Fig. 33.12) is the northern feather mite, so-called because it occurs mostly in temperate and north temperate as well as in subtropical areas. It has been reported from 22 species of birds, including domesticated poultry and English sparrows, also from rats, and accidentally from man. It resembles the common red mite, but differs from it in that it occurs both on birds and on their surroundings more or less continuously, even during the daytime. When infested birds are handled, the mites quickly crawl over the examiner's hands and arms. Parting the feathers reveals the mites, their eggs, cast-off skins, and excrement on the body surface and feathers, making the bird appear soiled. Feather mites are vicious blood suckers. They also cause
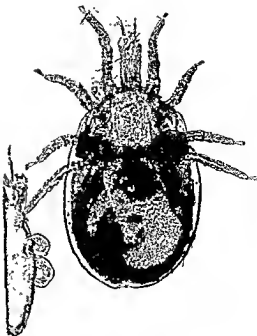
FIG. 33.12 — *Ornithonyssus sylviarum*. The northern feather mite, female. X73. (Benbrook and Sloss.)

scabs to form, thus injuring the appearance of dressed poultry (Payne, 1930).

Control of feather mites. This involves both the birds *and* their surroundings. Among the methods for control were those proposed by Payne (1929), Cutright (1929), Povar (1946), Richter and Insko (1948b), Furman *et al.* (1953), Moore and Schwardt (1954), Edgar and McAnnally (1955), Reid *et al.* (1956), Hoffman (1956), and Furman and Coates (1957).

For reports on more recent methods for controlling the northern feather mite, reference is made to Linkfield and Reid (1958), Kraemer (1959), Kraemer and Furman (1959), Rodriguez and Riehl (1959, 1960b), Bigley *et al.* (1960), Knapp and Krause (1960), Simco *et al.* (1962b), and the U.S. Department of Agriculture (1962b).

Specific treatments to control the northern feather mite are found on page 926 under Control in General.
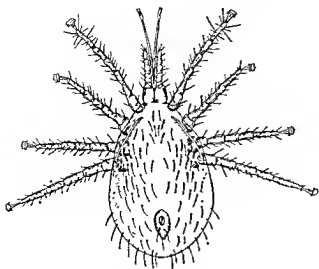
FIG. 33.13 — Ornithonyssus bursa. The tropical feather mite, female. Enlarged. (Reis and Nobrega.)

Tropical Feather Mite

Ornithonyssus bursa (Fig. 33.13), the tropical feather mite, is closely allied to the northern feather mite. It is more prevalent in warm or hot climates although it has been found in the northern United States. Control is accomplished in much the same manner as for the northern feather mite, particular attention being paid to the birds themselves and their nests. See page 926 for specific control methods under Control in General.

Chiggers

Trombicula alfreddugèsi, a chigger, is the six-legged larval stage (Fig. 33.14) of a mite that may infest the skin surface of various bird hosts as well as mammals, including man. The adults are not parasitic. Unfed chigger larvae are from 0.1 to 0.45 mm. in diameter, hence hardly visible unless they are engorged, when they appear as minute red dots. The adults breed on the ground, especially along fence rows or in undisturbed wooded or brushy areas. The larvae attach to the skin, often in groups, by means of their mouthparts, and inject a highly irritant substance into the wound. There follows a liquefaction of the skin which provides the larval chigger with food (Wharton and Fuller, 1952; Jones,

1950). Itching vesicles or even abscesses may form at the points of attachment, surrounded by a zone of hyperemia and edema. Apparently a toxemia may occur as is indicated by the mortality that follows infestation of chicks, especially quail.

Trombicula batatas is a tropical chigger of importance from southern United States to Brazil according to Michener (1916), Doetschman and Furman (1949), and Baker *et al.* (1956). It attacks chickens, tur-
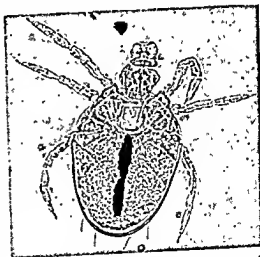
FIG. 33.14 — Trombicula alfreddugèsi larva. A chigger. X130. (Benbrook and Sloss.)

keys, wild birds, man, and domesticated mammals.

Neoschöngastia americana, the chicken chigger, is an important pest of chickens (Ewing, 1929) and turkeys in the southern United States. It also infests quail. Wharton is quoted by Baker *et al.* (1956) as stating that, in North and South Carolina, lesions on the skin of turkeys have to be removed before marketing the carcasses.

Acomatacarus galli, a chigger usually found on the rabbit, rat, and mouse, may be a pest of the chicken, according to Loomis (1956).

Control of chiggers. Most of the recommendations for poultry are based upon those used to combat chiggers on man, or for chiggers on the ground. The older preventive methods, such as the use of powdered sulfur, sulfur ointment, balsam of Peru, and phenol have more or less been replaced by dimethyl phthalate, dimethyl carbate, ethyl hexanediol, and benzyl benzoate. Efficient as they are on man, their use on flocks of birds appears to be impractically expensive at present

Sprays are useful for controlling chiggers on the ground. The Iowa State University Cooperative Extension Service (1964) recommends a spray containing 50 to 57 per cent malathion emulsifiable concentrate at a dilution of one-half pint to 10 gallons of water for 1,000 square feet of vegetation.

Reference is also made to a leaflet on chigger control from the U.S. Department of Agriculture (1956).

Scaly-Leg Mite

Knemidocoptes mutans (Fig. 33.15), the scaly-leg mite, and related species are of common occurrence on various birds, particularly the older ones that should ordinarily be culled from flocks. The mites are almost spherical in shape, short-legged, and adult females are about 0.5 mm. in diameter. The male is less than half the size of the female, and the legs are longer. Lesions are produced on the unfeathered portions of the host's legs and occasionally on the skin of the comb and wattles. Tunnels are bored into the epithelium, causing
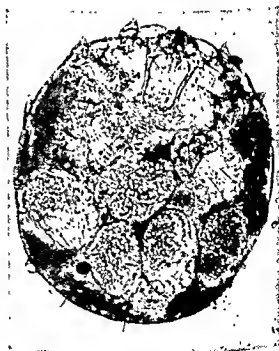
FIG. 33.15 – Knemidocoptes mutans. The scaly-leg mite, gravid female. ×150. (Benbrook and Sloss.)

FIG. 33.16 — Lesions produced by Knemidocoptes mutans, the scaly-leg mite. (Benbrook and Sloss.)

proliferation and the formation of scales and crusts (Fig. 33.16). This type of mite invasion of birds corresponds to sarcoptic mange of mammals. Affected birds may be crippled if the infestation is severe. The mites pass through their entire life cycle in the skin. Transmission to uninfested birds progresses slowly by contact with those infested and with their surroundings.

Control of scaly-leg mites should begin by culling or by isolating the affected birds. Additions to the flock should be inspected for lesions. Houses should be cleaned frequently, especially the roosts, which should be sprayed as recommended for red mites.

For individual treatment of birds having scaly-leg, Griffiths and O'Rourke (1950) recommended benzene hexachloride (BHC) as a 0.1 per cent emulsion to be applied to each leg for 30 seconds with a nail brush. Its odor may be transmitted to eggs. Cleland (1953) used 0.5 per cent by

weight of lindane (gamma BHC) in raw linseed oil, which has a less objectionable odor. It may be necessary to repeat scaly-leg therapy at two-week intervals.

Depluming Mite

Knemidocoptes laevis var. gallinae, the depluming or body mange mite, resembles the scaly-leg mite in general structure although it is smaller, the adult female being about 0.3 mm. in diameter. It invades the feathered areas of the epidermis of chickens, pigeons, and pheasants, especially around the feather bases. Intense irritation induces the host to pull out body feathers. The mites are more prevalent in the spring and summer at which time the infestation may spread rapidly by contact. Depluming mites produce injury by interfering with the control of body heat. Some of the affected birds will lose weight and show lowered production.

Control is not easily accomplished. Prompt isolation of affected birds and disinfestation of houses as recommended for red mites should come first. Treatments recommended for individual birds include:

A. Dipping in sulfur 2 ounces, soap 1 ounce, and warm water 1 gallon. This mixture should be thoroughly soaked into the skin and especially into the feet of cockerels. If lice are also present, there may be added to the dip, sodium fluoride or sodium fluosilicate 1 ounce.

B. Ointments may be used, consisting of sulfur 1 part, petrolatum 4 parts; or caraway oil 1 part, petrolatum 5 parts.

C. Moisten affected areas with soapy water, then apply powdered pyrethrum or powdered sulfur with the aid of a powder blower.

D. Possibly 0.1 per cent lindane emulsion may control this mite according to Baker et al. (1956).

Air-Sac Mite

Cytodites nudus (Fig. 33.17) is a mite that has learned to live as an internal parasite of the respiratory system, includ-

FIG. 33.17 — Cytodites nudus. The air-sac mite, male. ×100 (Benbrook and Sloss.)

ing the bronchi, lungs, air-sacs, and bone cavities connected therewith in birds. Air-sac mites have been found in chickens, turkeys, pheasants, and pigeons from many parts of the world. Although not ol common occurrence, these mites are often overlooked because of their small size and peculiar habitat.

The adult female mites are whitish specks, measuring about 0 5 to 0.6 mm. in length and 0.4 mm. in width. No details are known of the life cycle, although the usual speculation is that the mites lay larvae in the lower air passages, that these are coughed up and probably are swallowed, reaching the ground in the droppings. The mode of infection is not known.

There is considerable conflict among observers as to the damage done by air-sac mites. Some are of the opinion that they are practically harmless because their presence has been noted in apparently healthy birds. Others state that the mites are responsible for emaciation, peritonitis, pneumonia, obstruction of air passages, and that they are predisposing factors for tuberculosis. Heavy invasions have definitely been associated with weakness and grave loss in weight, so that the affected birds resemble clinical cases of tuberculosis.

Close inspection of the opened cadaver of an affected bird soon after death will show whitish dots moving slowly over the transparent air-sac surfaces. Identification may easily be made by placing mites in a drop of water on a slide, applying a cover glass, and examining under magnification of 100 diameters. Little information has been published as to the control of air-sac mites. Most writers recommend the destruction of the cadavers of affected birds, followed by disinfection and cleaning of the poultry house. Baker et al. (1956) suggest the possible use of a dust inhalant containing DDT or BHC as a control measure.

Subcutaneous Mite

Laminosioptes cysticola (Fig. 33.18), the subcutaneous or flesh mite, is another example of an originally external parasite invading deeper tissues. It has been
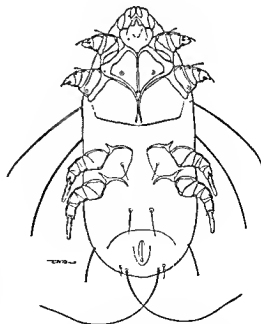
FIG. 33.18 — Laminosioptes cysticola. The subcutaneous mite, female. ×376. (Hirst.)

reported mainly from chickens, also from turkeys, pheasants, geese, and pigeons in many parts of the world.

Perhaps it normally is a parasite of the surface or upper layers of skin cells. However, it is most frequently noticed in the loose subcutaneous connective tissue. It has even been reported as occurring in the muscles, abdominal viscera, lungs (pigeons), and on the peritoneum.

Ordinarily, subcutaneous mites do not appear to influence the health of infested birds, although the lesions produced may make carcasses unpalatable as food for man.

The female mite measures about 0.25 to 0.26 mm. long by about 0.11 mm. wide. A distinctive feature is the transverse constriction around the body posterior to the second pair of legs. The life cycle is unknown except that the female lays embryonated eggs. Neveu-Lemaire (1938) states that the mite will pass through all stages of its development even in the deeper tissues of the host.

Attention is most often called to subcutaneous mites by the occurrence of yellowish nodules up to several millimeters in diameter in the subcutis. These areas are often mistaken for tuberculous lesions. The nodules appear to be caseo-calcareous deposits formed by the bird so as to enclose the mites after they die in the tissues. Large numbers of nodules are most often found in aged emaciated birds. Kasparek (1907) reported *L. cysticola* in pigeons in which the mites were surrounded by nodules in the lungs, causing death.

Perhaps more careful examination of the skin and subcutis of birds under a dissecting microscope might reveal the presence of this parasite more frequently. Otherwise, diagnosis will depend upon finding the characteristic nodular lesions and by seeing the mites or their remains in nodules that have been crushed under a cover glass in a drop of acidulated water, according to Lindquist and Belding (1949).

Apparently no attempt has been made to control subcutaneous mites except by the destruction of affected birds.

Other Mites

Following is a brief discussion of the less frequently reported mites of poultry. They variously damage the skin, eat feathers, or damage feather quills.

Epidermoptes bilobatus (Fig. 33.19) is a skin mite frequently reported from Europe and more rarely from South and North America, according to James *et al.* (1930). It occurs on chickens and apparently may or may not produce lesions. The adult female is about 0.17 to 0.22 mm. long. When lesions are produced, they consist first of a fine scaly dermatitis. This may be followed by the formation of thick, brownish, sharply-edged scabs. Neveu-Lemaire (1938), of France, suggests that the more severe lesions may be due partly to a concomitant fungous infection by *Lophophyton gallinae*; also that birds affected with scaly-leg mites often have depluming mites at the same time. Epidermoptic scabies may at times result in emaciation and even death. Pruritus is a common symptom.

Treatment of infested birds is recommended as for depluming scabies. In addition, Neveu-Lemaire (1938) suggests the use of balsam of Peru and alcohol, equal parts, applied to the skin.

Rivoltasia bifurcata, a feather-eating mite similar to *Epidermoptes*, has been reported on chickens in Europe, by Reis (1939) in Brazil, and by Bushnell and Twiehaus (1945) in a Kansas publication. In size it is said to be 0.25 by 0.15 mm. Only slight damage to feathers has been noted.

Syringophilus bipectinatus is commonly known as a quill mite. Originally described in Europe in 1880, it was not until 1932 that it was reported in the United States by Rebrassier and Martin (Ohio) from the chicken, turkey, and golden pheasant. Schwabe (1956) found this mite on chickens in New Jersey. Hwang (1959) reported *S. bipectinatus* on chickens in Mary-
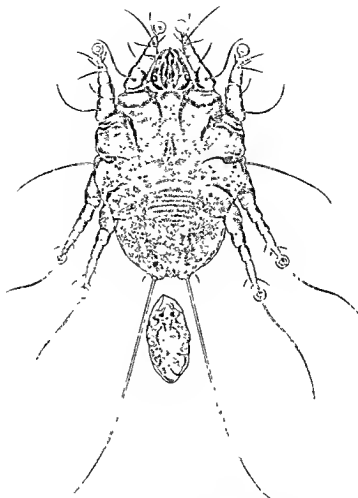
FIG. 33.19 — Epidermoptes bilobatus. The epidermoptic scabies mite, female. ×200.
(Reis and Nobrega.)

land and Pennsylvania. His paper is illustrated by photographs of affected and healthy quills; also by photomicrographs of adult female and male mites. He also differentiates this mite from *Syringophilus columbae* of pigeons. A similar mite, *S. columbae*, has been described from pigeons by Hirst (1922). Lavoipierre (1953) had previously described the female and the male pigeon quill mite. Wild birds harbor related species. *S. bipectinatus* females measure up to 0.9 mm. in length and to 0.15 mm. in width. The mites appear to cause partial or complete loss of feathers. The remaining quill stumps contain a powdery material in which the mites may be detected under low power magnification. No specific method for control has as yet been described. It would appear advisable to dispose of affected birds, then disinfect and clean their quarters.

Falculifer rostratus (Fig. 33.20), another feather-damaging mite, occurs principally between the barbs of the large wing feathers of pigeons. Although reported mainly in Europe, this mite has been noted
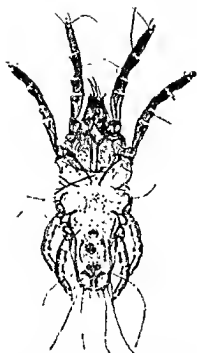
FIG. 33.20 — Falculifer rostratus. A feather mite of pigeons, male. ×68. (Reis and Nobrega.)

in the United States, and it may be more or less widespread. In size, the mite may be 0.8 mm. long (Hollander, 1956). Levi (1957) states that he has not found the feathers to be harmed. There is some evidence that the nymphal stage of the mite may occur in the subcutis or the internal organs.

Pillers (1927) and others recommend that infested pigeons be fumigated with sulfur dioxide gas. This appears to be a tedious and rather dangerous procedure. Yager and Gleiser (1946) treated a group of Signal Corps pigeons for infection by Falculifer rostratus, using 10 per cent DDT in talc. Slight control was achieved in 24 hours, with great reduction of the mites in 5 days, and there was no evidence of toxicity to the pigeons.

Freyana (Microspalax) chanayi, also a feather-inhabiting mite, has been reported from turkeys in Maryland by Chapin (1925). Its prevalence in Texas and Louisiana is mentioned by Bushnell and Twiehaus (1945). These mites congregate in the grooves on the under sides of the shafts of the wing feathers.

Megninia gallinulae is a rarely reported mite, according to Wickware (1921), in Canada. Apparently it is associated with loss of scales from the lower legs of chickens and with a crusty dermatitis in the head region. Neveu-Lemaire (1938) lists a similar species, M. cubitalis, from the body of chickens and turkeys in Europe and North America. The latter species is about 0.4 mm. long. Alicata et al. (1946) were able to reduce drastically the numbers of body mites, Megninia cubitalis, by applying 10 per cent Lethane A-70, NH dust, 5 per cent DDT, undiluted sodium fluoride, or undiluted sodium fluosilicate to Hawaiian chickens. They also found that good control could be obtained for the wing mite, Pterolichus obtusus, by using 10 per cent Lethane A-70 or by undiluted NH dust.

Levi (1957) states that pigeons in South Carolina may have the feathers of the neck and body infested by Megninia columbae.

Nasal Mites

Neonyssus columbae, a nasal mite of pigeons in Texas, was first described by Crossley (1950). It is about 0.7 mm. in length. No further information is available regarding it.

Neonyssus melloi, also a nasal mite of pigeons, was found by Crossley (1952) in Texas and Kansas. Formerly it had been first reported in Brazil.

Speleognathus striatus, a third nasal mite of pigeons, was first found in Texas by Crossley (1952). The mite is white and to 0.5 mm. in length. Clark (1957) reviewed the avian nasal mites belonging to the family Speleognathidae, stating that no pronounced lesions had been found in the hosts.

In addition to the mites listed, numerous other species have been noted on birds in various parts of the world. Some of these no doubt will be found invading

domestic poultry. For the present they may be considered of minor importance.

TICKS

Although ticks are quite important parasites of mammals, they are of relatively minor significance to birds.

Both ticks and mites are invertebrate arthropods of the class ARACHNIDA, order ACARINA. The ticks constitute a blood-sucking superfamily, namely the Ixodoidea. They are distinguished from the mites by being usually larger (to 15 mm. in length) and by the presence of a pair of respiratory openings, one on each side of the leathery abdomen. These openings, called spiracles or stigmal plates are situated either between the bases of the last two pairs of legs (family Argasidae) or posterior to the last pair (family Ixodidae).

A typical tick life cycle includes the egg, the larva (seed tick), the nymph, and the adult stages. After engorging with blood, the female tick drops from the host to hide in soil, humus, litter, tree bark, or crevices during the pre-ovulation and egg-laying periods. From several hundred to several thousand eggs are laid. This takes weeks to months after which the female tick dies. The male previously had died following copulation. After an incubation period, the minute six-legged larvae (seed ticks) emerge from the eggshells and await contact with a suitable host. Feeding on blood is followed by molting of the larval skin and emergence of the eight-legged nymphs that resemble the adults except for maturity of the reproductive organs. One or more additional molts follow before the adult female and male ticks fully develop.

The total length of the tick life cycle varies greatly, depending upon the species of tick, availability of suitable hosts, and climatic conditions. Some ticks complete the cycle within 6 weeks; others may require 2 years. In general, warmth and relative dryness favor tick development, although many species can withstand extremely cold weather. On vacated premises the adults, especially, may remain alive for many months or even several years.

Disease caused by ticks may be of three general types. Foremost is the loss of host blood, which may result fatally. Secondly, there must be considered loss in production, no doubt associated with blood loss, but also possibly due to tick-produced toxic substances. Thirdly, ticks in general are notorious transmitters of other parasites, such as those of avian spirochaetosis, tularemia, babesiosis, anaplasmosis, dirofilariasis, encephalomyelitis, and certain rickettsial diseases, notably Rocky Mountain spotted fever. The first two diseases are of particular interest to poultry raisers. Other avian diseases may be associated with tick transmission as has been suggested by Brown and Cross (1941) for avian leukosis.

Few species of ticks are host-specific, and those found on birds are no exception. The principal ticks reported from birds in North America are:

Argas persicus, the chicken tick that also occurs on geese, ducks, turkeys, guinea fowl, ostriches, pigeons, canaries, various wild birds, and rarely on cattle and on man (Cooley and Kohls, 1944).

Haemaphysalis leporis-palustris, the rabbit tick, is found mainly on rabbits and hares, also on dogs, cats, horses, and rarely on man. It may infest the chicken, quail, and various other wild birds.

Haemaphysalis chordeilis, a wild bird tick, has also been recorded from the turkey in North America, as well as from various domesticated mammals and man (Cooley, 1946).

Amblyomma americanum, the lone star tick, has been found on chickens and turkeys although it is usually a parasite of the horse, cattle, many other mammals, and several wild bird hosts.

Amblyomma tuberculatum, the gopher-tortoise tick, has been found on the chicken. Other hosts include cattle and the dog, several species of wild birds, and the gopher-tortoise.

Ornithodoros hermsi, is one of the ticks that may transmit a spirochete of relaps-

ing fever to man. It has also been found on "fowl," bat, chipmunk, deer, and mouse.

The Fowl Tick

Argas persicus, the fowl tick (Fig 33.21), is the most important tick parasite of birds. Among its many common names are chicken tick, blue "bug," tampan, and adobe tick. In the United States it is distributed mainly in those states along the Gulf of Mexico and the Mexican border. It is also established in many other tropical and temperate areas of the world. Although primarily a parasite of birds, it may be found on mammals. In North America it has been reported from the following hosts: chicken, duck, dove, hawk, magpie, owl, quail, sparrow, thrush, vulture, and wild turkey; also from cattle, dog, and man.

The mature, blood-engorged female measures about 10 mm. in length and about 6 mm. in width, the mature male being about half that size. This tick is relatively easily recognized by its flattened ovoid shape and reddish-brown color. There is no scutum or dorsal shield, which thus distinguishes it as belonging to the tick family Argasidae. The mouthparts are on the ventral anterior surface, hidden from above by the projecting body.

The female fowl tick may lay a total of 700 eggs at several layings, between which she seeks a host for a meal of blood. The eggs are laid in sheltered crevices, including the bark of trees. They hatch from 10 days in warm weather to 3 months during cool periods. The almost microscopic larvae or seed ticks immediately seek a host, although they may live for several months without eating. After feeding on blood for several days, the larvae leave the host for a hiding place nearby, and generally in 1 to 9 days they reach the nymphal stage. Nymphs may do without food for as long as 15 months. However, if a host is available, the nymphs feed during a night, then hide for 10 or 12 days, shed their skins, and reach a second nymphal stage. After about an hour's feeding at night and about a week in hiding, the adult ticks emerge from the nymphal skins, now ready to engorge with blood and reproduce over a period of about 30 days. The adult fowl tick may live for more than 2 years in an unfed state. Loomis (1961), working under laboratory conditions, found the complete life cycle of *Argas persicus* to be from 7 to 8 weeks.

Birds suffer to the greatest degree from attacks of these ticks during the warmer,

FIG. 33.21 – *Argas persicus*. The fowl tick or blue "bug," female, nearly engorged with blood. Dorsal and ventral views. (U.S.D.A., Bur. Entom. and Plant Quaran.)

dryer seasons of the year. The loss of blood may reach the proportions of a fatal anemia. At least there may be expected to be emaciation, weakness, slow growth, and lowered production. Ruffled feathers, poor appetite, and diarrhea are symptoms suggesting tick infestation. Turkeys usually suffer even more than chickens, and recently hatched poults and chicks show the highest mortality

The fowl tick is capable of transmitting a spirochete from the blood of infected birds to that of susceptible birds in many parts of the world. This organism, *Borrelia anserina*, is highly pathogenic. Burroughs (1947) claimed to be the first to find tick-borne avian spirochaetosis in the United States. He allowed fowl ticks (*Argus persicus*) to feed on an apparently normal chicken, which, in 6 days, was positive for *Borrelia anserina* in blood smears. Apparently Brazil is the area nearest to the United States in which avian spirochaetosis commonly occurs. Rokey and Snell (1961) described epizootics of avian spirochaetosis in Arizona associated with infestations by the fowl tick. *Argus persicus* may also act as a vector of *Aegyptianella pullorum*, an erythrocyte-invading protozoan (piroplasm) of birds. The disease thus produced, aegyptianellosis, has not been reported from the Americas. Brown and Cross (1941) found evidence that fowl ticks are agents for the transmission of the virus of fowl paralysis. Howell

et al. (1943) reported that occasionally the fowl tick may transmit the protozoan parasite of anaplasmosis of cattle. Micks (1951) described methods for the laboratory rearing of the common fowl tick.

Control of the fowl tick. This is difficult because the ticks are found on their hosts for about a week and then they hide in the birds' surroundings in order to complete the life cycle.

Among the previous reports on control are those of Bishopp (1911), Dove (1915), Edgar *et al.* (1953), Furman and Weinmann (1956), Lapage (1962), Le Roux (1956), also Rodriguez and Richl (1956, 1957b).

At the present time, malathion and Sevin are recommended as sprays for the hiding places of fowl ticks and red mites. Concise directions for their use are given on page 926 under Control in General. Control methods are also described in a leaflet from the U.S. Department of Agriculture (1962a).

Tickicides should be used according to the instructions of the manufacturer or distributor, keeping in mind their potential toxicity to man and animals.

Other methods for the control of fowl ticks include the use of metal construction, the elimination of tree roosting, using roosts suspended from the ceiling. Frequent inspection is necessary in order to combat ticks before their number has increased to a harmful extent.

REFERENCES

Abbott, W. S.: 1920 Results of experiments with miscellaneous substances against chicken lice and the dog flea. U.S.D.A., Bul. 888.

Albert, T. F.: 1962 The effect of DDT on the sperm production of the domestic fowl. Auk 79:104

Alicata, J. E.: 1942. Experimental transmission of endemic typhus fever by the sticktight flea. *Echidnophaga gallinacea*. Jour. Wash. Acad. Sci. 32:57.

————: 1948. A method of collecting ectoparasites from birds. Univ. Hawaii Agr. Exper. Sta. Biennial Rep., p. 99.

————, Holdaway, F. G., Quisenberry, J. H., and Jensen, D. D.: 1946. Observations on the comparative efficacy of certain old and new insecticides in the control of lice and mites of chickens. Poultry Sci. 25:376.

————, Kartman, L., and Fisher, H. I.: 1948. Wild birds as possible carriers of poultry parasites. Univ. Hawaii Agr. Exper. Sta., Biennial Rep., p. 104.

————, Kartman, L., Nishida, T., and Palafox, A. L.: 1947. Efficacy of certain sprays in control of lice and mites of chickens. Jour. Econ. Entom. 40,922.

Anderson, R. C.: 1956. The life cycle and seasonal transmission of *Ornithofilaria fallisensis* Anderson, a parasite of domestic and wild ducks. Canad. Jour. Zool. 34:485.

Annand, P. N., and Members of Staff: 1944. *Tests conducted by the Bureau of Entomology and Plant Quarantine to appraise the usefulness of DDT as an insecticide.* Jour. Econ. Entom. 37:125.

Ansari, M. A. R.: 1955. Synoptic table for the determination of Mallophaga infesting domestic fowl (*Gallus gallus domesticus*), Indian Jour. Entom. 17:245.

Arnold, H. L., Jr., and Arnold, H. L., Sr.: 1948. *The diagnosis and management of bird-mite bites (in man).* Proc. Staff Meet. Clinic, Honolulu. 9:41.

Back, E. A., and Cotton, R. T.: 1938. Stored grain pests. (Revised.) U.S.D.A. Farmers' Bul. 1260. (Beetles and beetle larvae.)

Baker, A. D.: 1933. Some studies of the dipterous fauna of the poultry yard in Quebec in relation to parasitic troubles. Poultry Sci. 12:42.

Baker, E. W., Evans, T. M., Gould, D. J., Hull, W. B., and Keegan, H. L.: 1956 A Manual of Parasitic Mites of Medical or Economic Importance. National Pest Control Assn., Inc., New York. 170 pp.

———, and Wharton, G. W.: 1952. An Introduction to Acarology. The Macmillan Co., New York. 465 pp.

Barger, E. H., Card, L. E., and Pomeroy, B. S.: 1958. Diseases and Parasites of Poultry. 5th ed. Lea and Febiger, Philadelphia. 408 pp

Barnes, S.: 1945. The residual toxicity of DDT to bedbugs (*Cimex lectularius*). Lond. Sch. of Trop. Hyg., Ministry of Prod., Insecticides Development Panel. Brit. Apt. 1DP (45):226.

Beaudette, F. R.: 1946. Common mosquito-borne diseases of birds. Proc. 33rd Ann. Meet., New Jersey Mosquito Externi. Assn., p. 31.

Benbrook, E. A.: 1963. Outline of Parasites Reported for Domesticated Animals in North America. 6th ed. Iowa State University Press, Ames, Iowa. 240 pp.

———, and Sloss, M. W.: 1961. Veterinary Clinical Parasitology. 3rd ed. Iowa State University Press, Ames, Iowa. 240 pp., 288 illus.

Bequaert, J. C.: 1954. The Hippoboscidae or louse-flies (Diptera) of mammals and birds. Part II. Taxonomy, evolution and revision of American genera and species. Entom. Americana n.s. 34:1.

Bermde, W. H. J.: 1952. The chicken mite attacking children. Jour. Econ. Entom. 45:1098.

Bierer, B. W.: 1954 Buffalo gnats and Leucocytozoon infections of poultry, Vet. Med. 49:107, 115.

Bigland, C. H.: 1955. Chicken infestation with *Ceratophyllus niger* (black hen flea) in Alberta. Canad. Jour. Comp. Med. and Vet. Sci. 19.90.

Bigley, W. S., Roth, A. R., and Eddy, G. W.: 1960. Laboratory and field tests against mites and lice attacking poultry. Jour. Econ. Entom. 53:12.

Bishopp, F. C.: 1923. Limberneck of fowls produced by fly larvae. Jour. Parasit. 9.170.

———: 1929. The pigeon fly, an important pest of pigeons in the United States. Jour. Econ. Entom. 22.974.

———: 1933. Mosquitoes kill livestock. Science 77:115.

———: 1941. The fowl tick. U.S.D.A., Farmers' Bul. 1070 (revised).

———: 1942a. Poultry lice and their control. U.S.D.A., Yearbook, p. 1048.

———: 1942b. The pigeon fly. U.S.D.A., Yearbook, p. 1072.

———, and Wagner, R. D: 1931. Nicotine in the control of ectoparasites of poultry. Jour. Econ. Entom. 24:56.

Blacklock, D.: 1912. On the resistance of *Cimex lectularius* to various reagents, powders, liquids, and gases. Annals Trop. Med. and Parasit. 6:415.

Boyt, R. H.: 1937. *Dermanyssus* and *Liponyssus avium* et *gallinae* attacking man. Brit. Jour. Dermatol. 49.66.

Brennan, J. M.: 1951. Two new species of Neoschöngastia with a key to the species of the world (Acarina: Trombiculidae). Jour. Parasit. 37:577.

Brody, A. L.: 1936. The transmission of fowl pox. (By mosquitoes) Cornell Univ. Agr. Exper. Sta., Memoir 195.

Brown, A. W. A.: 1951. Insect Control by Chemicals. John Wiley and Sons, New York. 817 pp.

Brown, J. C., and Cross, J. C.: 1941. A probable agent for the transmission of fowl paralysis. (Fowl tick.) Science 93.528.

Brown, J. H.: 1953. A chicken mite infestation in a hospital. Jour. Econ. Entom. 46:900.

Burroughs, A. L.: 1947. Fowl spirochaetosis transmitted by *Argas persicus* (Oken). 1818 from Texas. Science 105:577.

Bushnell, L. D., and Twiehaus, M. J.: 1945. Poultry disease, their prevention and control. Kans. Agr. Exper. Sta. Bul. 326.

Cameron, D.: 1938. The northern fowl mite (*Liponyssus sylviarum*). Investigations at Macdonald College, Quebec, with a summary of previous work. Canad. Jour. Rex. 16.230 (Sec. D).

Carpenter, C. D.: 1931. The use of mosune and its compounds for the control of poultry parasites. Jour. Am. Vet. Med. Assn. 78.651.

Cassamagnaghi, A., Jr., Bianchi Bategue, A., and Ferrando, H.: 1950. Sobre las endoacariasis de las gallinas y palomas domesticas. Direc. Ganaderia Bol. Mens. (Uruguay) 31:273.

Chamberlain, R. W., and Sikes, R. K.: 1950. Laboratory rearing methods for three common species of bird mites. Jour. Parasit. 36:461.

Chamberlain, R. W., and Sikes, R K.: 1955. Laboratory investigations on the role of bird mites in the transmission of eastern and western equine encephalomyelitis. Am. Jour. Trop. Med. and Hyg. 4:106.

Chapin, E. A.: 1925. *Freyana* (*Microspalax*) *chaneyi* from a turkey, *Meleagris gallopavo.* Jour. Parasit. 12:113.

Clark, G. M.: 1957. Observations on the acarine family Speleognathidae, including two previously unreported forms in native game birds. Jour. Parasit. 43, Sec. 2:34.

Cleland, J. W.: 1933. A preliminary note on the control of *Cnemidocoptes mutans* Robin. New Zealand Entom. 1.17.

Cooney, G. R.· 1931. On the biology of the pigeon fly, *Pseudolynchia maura* Bigot. Parasit. 23 525.

Cooley, R. A.: 1946. The genera *Boophilus, Rhipicephalus* and *Haemaphysalis* (Ixodidae) of the New World. Nat. Inst. Health Bul. 187.
———, and Kohls, G. M.: 1944. The Argasidae of North America, Central America, and Cuba. The Univ. Press, Notre Dame.

Cotton, R. T., and St. George, R. A.: 1929. The meal worms. (Beetles and beetle larvae.) U.S.D.A, Tech Bul. 95

Creel, R. H., and Faget, F. M.: 1916. Cyanide gas for the destruction of insects. U.S. Pub. Health Service, Reprint 343 from Pub. Health Reps., p. 1464.

Creighton, J. T., Dekle, G. W., and Russell, J.: 1943. The use of sulfur and sulfur compounds in the control of poultry lice. Jour. Econ. Entom. 36:413
———, Hetrick, L. A., Hunt, P. J., and Duncan, D. U.: 1947. The application of chlorinated hydrocarbons to the soil and roosts effectively controls lice of poultry. Poultry Sci. 26.674.

Cross, H F: 1962 In vivo studies of tissue reaction in chicks resulting from the feeding by larvae of *Trombicula splendens.* Jour Econ. Entom. 55:22, 27.
———, and Tolger, G. G.: 1956. The use of acaricides on cats and birds. Jour. Am. Vet. Med. Assn. 129 65.

Crosley, D A., Jr.: 1950. A new species of nasal mite, *Neonyssus columbae,* from the pigeon. (Acarina, Mesostigmata, Rhinonyssidae). Entom Soc. Wash. Proc 52:309.
———. 1952 Two new nasal mites from columbiform birds. Jour. Parasit. 38:385.

Crutchfield, C M., and Hixson, H. 1943. Food habits of several species of poultry lice, with special reference to blood consumption. Fla. Entom. 26.63.

Cunningham, H. B., Little, C. D., Edgar, S. A., and Eden, W. G.: 1955. Species and relative abundance of lice collected from chicken manure in Alabama. Jour. Econ. Entom. 48.620.

Cutright, C. R.: 1929. A valuable aid in the control of the feather mite, *Liponyssus sylvarum.* Jour. Econ. Entom. 22:422.

Davidson, W. M.: 1924. Results of experiments with miscellaneous substances against the chicken mite. U.S.D.A., Dept. Bul. 1228

Davis, W. A.: 1940 A study of birds and mosquitoes as hosts for the virus of eastern equine encephalomyelitis. Am. Jour. Hyg., Sec. C. 32.45.

Deakin, A., and Robertson, G.: 1933. Effect of mercurial ointment on hatchability. Poultry Sci. 12:378

de Olivera Castro, G. M.· 1930. The transmission of epithelioma contagiosa by mosquitoes (trans. title). Compt rend Soc. de biol. 105 316.

de Ong, E. R.· 1956 Chemistry and Uses of Pesticides. 2nd ed. Reinhold Publ. Corp., New York. 334 pp.
———. 1960. Chemical and Natural Control of Pests Reinhold Publ. Corp., New York. 252

de Zayas, F.: 1941 Los malophagos de las aves domesticas en Cuba. Univ. Havana, Mem. de la Soc. Cub. de Hist Nat 15-201.

Dietrich, A.: 1925. *Laminosioptes cysticola* und *Cytoleichus sarcoptoides* bei Hühnern. Berliner tierärztl. Wochenschr. 41-466.

Doetschman, W H., and Furman, D. P. 1949. A tropical chigger, *Eutrombicula batatas* (Linn) attacking man in California Am Jour. Trop. Med. 29.605.

Dorough, H. W., Brady, U E., Jr, Timmerman, J. A., Jr., and Arthur, B. W.: 1961. Residues in tissues and eggs of poultry dusted with Co-Ral (Bayer 21/199). Jour. Econ. Entom. 54.25.

Dove, W. E.: 1945. Summary of DDT experiments on insects that affect man and animals. U.S.D.A., Bur. Entom. and Plant Quar., Mimeo. Cir. E-673.

Dow, R. P., Reeves, W. C., and Bellamy, R. E.: 1957 Field tests of avian host preference of *Culex tarsalis* Coq. Am. Jour. Trop. Med. and Hyg. 6:294.

Downe, A. E. R., and Morrison, P. E.: 1957. Identification of blood meals of blackflies (Diptera: Simuliidae) attacking farm animals. Mosquito News 17:37.

Drake, C. J., and Jones, R. M.: 1930. The pigeon fly and pigeon malaria in Iowa. Iowa State Coll. Jour. Sci. 4 255.

Dubinin, W. B.: 1953. Analgesoidea. II. Families Epidermoptidae and Freyanidae (trans. title). Fauna SSSR 57:1. (Biol. Abst. 31 No. 36566, 1957.)

Eads, R. B.: 1946. Control of the sticktight flea on chickens. Jour. Econ. Entom. 39:659.

Eddie, B., Meyer, K. F., Lambrecht, F. L., and Furman, D. P.: 1962. Isolation of ornithosis bedsoniae from mites collected in turkey quarters and from chicken lice. Jour. Inf. Dis. 110:231.

Edgar, S. A.: 1953. A field study of the effect of black fly bites on egg production of laying hens. Poultry Sci. 32:779.

——, and King, D. F.: 1948. Comparative efficiency of several old and new insecticides in the control of lice on poultry and the effect of the body louse, *Eomenacanthus stramineus*, on egg production. Poultry Sci. 27:659.

——, and King, D. F.: 1950. Effect of the body louse, *Eomenacanthus stramineus*, on mature chickens. Poultry Sci. 29:214.

——, Little, C. D., and Herndon, J. F.: 1953. Control of the fowl tick, *Argas persicus* (Oken), on an Alabama poultry farm. Jour. Am. Vet. Med. Assn. 123:446.

——, and McAnnally, B. D.: 1955. Control of the northern feather mite, *Bdellonyssus sylviarum*, on chickens in cages and on litter. Poultry Sci. 34:91.

——, Walsh, W. L., and Johnson, L. W.: 1949. Comparative efficacy of several insecticides and methods of application in the control of lice on chickens. Poultry Sci. 28:520.

——, and Williams, O. M.: 1948. Effect of mosquitoes on poultry. Poultry Sci. 27:660.

——, Williams, O. M., and Hester, E. F.: 1951. Feeding habits of mosquitoes and their effect on poultry. Poultry. Sci. 30:911.

Emerson, K. C.: 1951. A list of Mallophaga from gallinaceous birds of North America. Jour. Wildlife Mgt. 15:193.

——: 1956. Mallophaga (chewing lice) occurring on the domestic chicken. Jour. Kans. Entom. Soc 29:63.

Emmel, M. W.: 1937. Sulfur in the control of external parasites of chickens. Preliminary report. Jour. Am. Vet. Med. Assn. 91:201.

——: 1942. Field experiments in the use of sulfur to control lice, fleas, and mites of chickens. Fla. Agr. Exper. Sta. Bul. 374.

Ewing, H. E.: 1911. The English sparrow as an agent in the dissemination of chicken and bird mites. Auk 28:335.

——: 1921. Studies on the biology and control of chiggers. U.S.D.A. Bul. 986

——: 1925. The dermanyssid mites of North America. Proc. U.S. Nat. Museum, 62, Art. 13:1.

——: 1929. Birds as hosts for the common chigger. Am. Nat. 63:94.

——: 1936. A short synopsis of the North American species of the mite genus Dermanyssus. Proc. Entom. Soc. Wash 58:47.

——: 1938. A key to the genera of chiggers (mite larvae of the subfamily Trombiculinae) with descriptions of new genera and species. Jour. Wash. Acad. Sci. 28:288.

——: 1944. The trombiculid mites (chigger mites) and their relation to disease. Jour. Parasit 30:339.

——, and Fox, I.: 1943. The fleas of North America. U.S.D.A. Misc. Publ. 500.

Fairchild, H. E., and Dahm, P. A.: 1955. Lice control on chickens with chlorinated hydrocarbon insecticides. Jour. Econ. Entom. 48:141.

Fallis, A. M., Anderson, R. C., and Bennett, G. F.: 1956. Further observations on the transmission and development of *Leucocytozoon simondi*. Canad. Jour. Zool. 34:389.

——, and Wood, D. M.: 1957. Biting midges (Diptera:Ceratopogonidae) as intermediate hosts for *Haemoproteus* of ducks. Canad. Jour. Zool. 35:425.

Floyd, E. H., and Tower, B. A.: 1956. Insecticide-impregnated litter for control of chicken body lice (*Eomenacanthus stramineus*) on poultry. Poultry Sci. 35:896.

Fox, Irving: 1940. Fleas of Eastern United States. Iowa State University Press, Ames, Iowa.

Frank, J. F.: 1953. A note on the experimental transmission of enterohepatitis of turkeys by arthropods. Canad. Jour. Comp. Med. and Vet. Sci. 17:250.

Frear, D. E. H.: 1955. Chemistry of the Pesticides. 3rd ed. D. Van Nostrand Co., New York.

——: 1961. Pesticide Index. College Science Publishers, State College, Pa. 193 pp.

——: 1962. Pesticide Handbook. 14th ed. College Science Publishers, State College, Pa. 303 pp.

Furman, D. P.: 1953. Comparative evaluation of control procedures against the northern fowl mite *Bdellonyssus sylviarum* (Can. and Fanz). Jour. Econ. Entom. 46:822.

——, and Pankowski, R. A.: 1949. Absorption of benzene hexachloride in poultry. Jour. Econ. Entom 42:980

——, and Coates, W. S.: 1957. Northern fowl mite control with malathion. Poultry Sci. 36:252.

——, Coates, W. S., and Rohrbacher, G. H.: 1953. Northern fowl mite control. Calif. Agr. 7 9.

——, and Pieper, G. R.: 1962. Systemic acaricidal effects of Sevin in poultry. Jour. Econ. Entom. 55:555.

——, and Weinmann, C. J.: 1956. Toxicity of malathion to poultry and their ectoparasites. Jour. Econ. Entom. 49:447.

Gallagher, B. A.: 1920. Rose chafer poisoning in chickens. Jour. Am. Vet. Med. Assn. 57:692.

Gibson, A.: 1930 Insect and other external parasites of poultry in Canada. Scient. Agr. 11:208.

Gless, E. E., and Raun, E. S.: 1958. Insecticidal control of the chicken body louse on range turkeys. Jour. Econ. Ent. 51:229.

——, and Raun, E. S.: 1959. Effects of chicken body louse infestation on egg production. Jour. Econ. Entom. 52:358.

Godfrey, G. F., Howell, D. E., and Graybill, F.: 1953. Effect of lindane on egg production. Poultry Sci. 32:183.

Goodman, J. G.: 1958. Effects of feeding boron to hens to prevent flies. Alabama Agr. Exper. Sta. Prog. Rep., Series 71. 2 pp.

Gould, G. E., and Moses, H. E.: 1951. Lesser mealworm infestation in a brooder house. Jour. Econ. Entom. 44.265.

Graeser, F. E.: 1943. Scabies in a turkey. Canad. Jour. Comp. Med. 7:15.

Griffiths, R. B., and O'Rourke, F. J.: 1950. Observations on the lesions caused by Cnemidocoptes mutans and their treatment, with special reference to the use of "Gammexane." Ann. Trop. Med. and Parasit. 44.93.

Guberlet, J. E., and Hotson, H. H.: 1940. A fly maggot attacking young birds, with observations on its life history. Murrelet 21:65

Guilhon, J.: 1952. Gale épidermoptique de la poule. Bul. Acad. Vet. France 25.83.

Hall, M. C.: 1929. Arthropods as intermediate hosts of helminths. Smithsonian Inst., Misc. Collections 81 1-77.

Hammon, W. McD., Reeves, W. C., Cunha, R., Espana, C., and Sather, G.: 1948. Isolation from wild bird mites (Liponyssus sylvarum) of a virus or mixture of viruses from which St. Louis and western equine encephalitis viruses have been obtained. Science 107:92.

Harding, W. C., Jr.: 1955. Malathion to control the northern fowl mite. Jour. Econ. Entom. 48 605

———. 1958 Lesser mealworms in a brooder house. Jour. Econ. Entom. 51:112.

———, and Quigley, D. J.: 1956. Litter treatment with malathion to control the chicken body louse. Jour. Econ. Entom. 49:806.

Hiatt, L.: 1938. A short note on the transmission of the fowl spirochete (Treponema anserinum) by red mite (Dermanyssus gallinae). Vet. Res. Rep, Dept. Agr., N. S. Wales, No. 7:74.

Harwood, P. D.: 1948. Benzene hexachloride and poultry meat. Science 107:113.

Hearle, E.: 1938 Insects and allied parasites injurious to livestock and poultry in Canada. Canad. Dept. Agr., Publ 604

Herman, C. M.: 1938a. Mosquito transmission of avian malaria parasites (Plasmodium circumflexum and P. cathemerium). Am Jour. Hyg., Sec. C, 27:345.

———. 1938b. Occurrence of larval and nymphal stages of the rabbit tick, Haemaphysalis leporis-palustris, on wild birds from Cape Cod. Bul. Brooklyn Entom. Soc 33:153.

Herms, W. B., and James, M. T.: 1961. Medical Entomology. 5th ed. The Macmillan Co. New York. 643 pp.

Hertel, M.: 1904. Über Geflügelcholera und Hühnerpest. Arb. a.d. kaiserl. Gesundheitsamt. 20-453

Hipolito, O., and [de] Freitas, M. G.: 1943. Notas ornitopatologicas. Observacões sôbre alguns acarinos parasitos de Gallus gallus domesticus, em Minas. Arq. Escola Superior Vet., Estado Minas Gerais (Brazil) 1.81.

Hirst, S.: 1922. Mites injurious to domestic animals. Brit. Museum, Econ. Series 13:1–107.

Hixon, E., and Muma, M. H.: 1947. Toxicity of certain insecticides to the chicken mite. Jour. Econ. Entom. 40 596.

Hocking, B.: 1953. Developments in the chemical control of black flies (Diptera:Simuliidae). Canad Jour. Agr. Sci 33 572.

Hoffman, R. A.: 1956 Control of the northern feather mite and two species of lice on poultry. Jour. Econ. Entom 49.847.

———. 1960. The control of poultry lice and mites with several organic insecticides. Jour. Econ. Entom. 53·160.

———. 1961 Experiments on the control of poultry lice. Jour. Econ. Entom. 54:1114.

———, and Drummond, R. O.: 1961. Control of lice on livestock and on parasites of poultry with General Chemical 4072. Jour. Econ. Entom. 54:1052.

———, and Monroe, R. E.: 1957. Further tests on the control of fly larvae in poultry and cattle manure. Jour. Econ. Entom. 50.515.

Holstad, M. S.: 1944. Recovery of Newcastle disease (pneumoencephalitis) virus from mites, Liponyssus sylvarum, after feeding upon Newcastle-infected chickens. Am. Jour. Vet. Res. 10.370.

Hollander, W. F.: 1956. Acarida of domestic pigeons. Trans. Am. Micro. Soc. 75:461.

Hopkins, G. H. E., and Clay, T.: 1952. A Check List of the Genera and Species of Mallophaga. Brit. Mus. (Nat Hist.) London. 362 pp.

Howell, D. E., Stiles, G. W., and Moe, L. H.: 1943. The fowl tick (Argas persicus), a new vector of anaplasmosis. Am. Jour. Vet. Res. 4:73.

Howitt, D. F., Dodge, H. R., Bishop, L. K., and Gorrie, R. H.: 1948. Virus of eastern equine encephalomyelitis isolated from chicken mites (Dermanyssus gallinae) and chicken lice (Eomenacanthus stramineus). Soc. Exper. Biol. and Med. Proc. 68.622.

Hoyle, W. L.: 1938. Transmission of poultry parasites by birds, with special reference to the "English" or house sparrow and chickens. Trans. Kans. Acad. Sci. 41:379.

Hubbard, C. A.: 1947. Fleas of Western North America. Iowa State University Press, Ames, Iowa.

Huff, C. G.: 1932. Further infectivity experiments with mosquitoes and bird malaria. Am. Jour. Hyg. 15:751.

Hungerford, T. G.: 1938. Field observations on spirochaetosis (*Spirochaeta anserina*) of poultry, transmitted by the red mite (*Dermanyssus avium*) in New South Wales. Vet. Res. Rep., Dept. Agr., N.S.W., No. 7:71.

Hwang, J. C.: 1959. Case report of the quill mite, *Syringophilus bipectinatus*, in poultry. Proc. Hel. Soc. Wash. 26:47.

Illingworth, J. F.: 1915. Notes on the habits and control of the chicken flea (*Echidnophaga gallinacea* Westwood). Jour. Econ. Entom. 8:492.

Iowa State University, Cooperative Extension Service: 1963. Summary of Iowa insect pest control recommendations for 1963. Publication IC-328 (revised). Ames, Iowa, 23 pp.

Ivey, M. C., Roberts, R. H., Mann, H. D., and Claborn, H. V.: 1961. Lindane residues in chicken and eggs following poultry house sprays. Jour. Econ. Entom. 54:487.

James, W. A., Graham, R., and Thorp, F.: 1930. Epidermoptic scabies in a hen. Jour. Am. Vet. Med. Assn. 76:63.

Johnson E. P., Underhill, G. W., Cox, J. A., and Threlkeld, W. L.: 1938. A blood protozoan of turkeys transmitted by *Simulium nigroparvum* (Twinn). Am. Jour. Hyg. 27:649.

Jones, B. M.: 1950. The penetration of the host tissue by the harvest mite, *Trombicula autumnalis* Shaw. Parasit. 40:247.

Jones, L. M.: 1965. Veterinary Pharmacology and Therapeutics. 3rd ed. Iowa State University Press, Ames, Iowa. 1085 pp.

Judd, W. W.: 1956. Dermatitis of humans caused by fowl mite, *Dermanyssus gallinae* (Deg) at London, Ontario. Canad. Entom. 88.109.

Kadner, C. G.: 1941. Pigeon malaria in California. Science 93:281.

Kartman, L.: 1949. Preliminary observations on the relation of nutrition to pediculosis of rats and chickens. Jour. Parasit. 35:367.

Kaschula, V. R.: 1950. "Scaly-leg" of the canary (*Serinus canaria* [L.]). Jour. So. Afr. Vet. Med. Assn. 21:17.

———, and Stephan, S. A. R.: 1947. Mites, hitherto unrecorded in South Africa, collected in Natal from fowls, pigeons, turkeys, guinea-fowls, wild birds, and rabbits. Onderstepoort Jour. Vet. Sci. and Anim. Ind. 22.51.

Kasparek, A.: 1907. Bericht über die 79. Versammlung Deutscher Naturforscher und Aerzte in Dresden. Deutsch. tierärztl. Wochenschr. 15 623. (*Laminosioptes cysticola*)

Kaura, R. L., and Iyer, S. G.: 1937. The occurrence of the air-sac mite, *Cytoleichus nudus* (Vinoll, 1870) in fowls in India. Indian Jour. Vet. Sci. and Anim. Husb. 7:299.

Kéler, S.: 1938. Übersicht über die gesamte Literatur der Mallophagen (1668–1938) Zeitschr. angew. Entom. 25:487.

Keller, J. C. and Gouck, H. K.: 1957. Small-plot tests for the control of chiggers. Jour. Econ. Entom. 50:141.

Kellogg, V. L.: 1900. A list of the biting lice (Mallophaga) taken from birds and mammals of North America. Proc. U.S. Nat. Museum 22:39.

Kitteiman, C. H., and Grundmann, A. W.: 1940. Equine encephalomyelitis virus isolated from naturally infected *Triatoma sanguisuga* Le Conte. Kans. Agr. Exper. Sta., Tech. Bul. 50.

Kligler, I. J., Muckenfuss, R. S., and Rivers, T. M.: 1929. Transmission of fowl pox by mosquitoes. Jour. Exper. Med. 49 649.

Knapp, F. W.: 1962. Co-Ral as a litter and nest dust to control the chicken body louse. Jour. Econ. Entom. 55:571.

———, and Krause, G. F.: 1960. Control of the northern fowl mite, *Ornithonyssus sylviarum* (C. and F.) with ronnel, Bayer L13/59 and Bayer 21/199. Jour. Econ. Entom. 53:4.

———, Terhaar, C. J., and Roan, C. C.: 1958. Dow ET-57 as a fly larvicide. Jour. Econ. Entom. 51:361.

Knipling, E. F., and Rainwater, H. T.: 1937. Species and incidence of dipterous larvae concerned in wound myiasis. Jour. Parasit. 23:451.

Köhnlein, J.: 1925. Die Vogelmilbe (*Dermanyssus avium*) und ihre Bekämpfung. Arch. f. wissen. Tierheilk. 53:144.

Kotlán, A.: 1923. Über die Blutaufnahme als Nahrung bei den Mallophagen. Zool. Anz. 56:231.

Kraemer, P.: 1959. Relative efficacy of several materials for control of poultry ectoparasites. Jour. Econ. Entom. 52:1195.

———, and Furman, D. P.: 1959. Systemic activity of Sevin in control of *Ornithonyssus sylviarum* (C. and F.). Jour. Econ. Entom. 52:170.

Kulash, W. M.: 1947. DDT for control of bedbugs in poultry houses. Poultry Sci. 26:44.

———, and Maxwell, J. M.: 1945. DDT and bedbugs in chicken houses. Jour. Econ. Entom. 38.606.

Lamson, G. H., Jr.: 1917. Mercurial ointment, an effective control of hen lice. Jour. Econ. Entom. 10:71.

———: 1922. The rose chafer as a cause of death of chickens. Storrs Agr. Exper. Sta., Bul. 110:117.

Lapage, G.: 1956. Veterinary Parasitology. Charles C Thomas, Springfield, Ill. 964 pp.

———: 1962. Mönnig's Veterinary Helminthology and Entomology. 5th ed. Williams and Wilkins Co., Baltimore. 597 pp.

Lavoipierre, M. M. J.: 1953. The undescribed male and female of the pigeon quill mite, *Syringophilus columbae* Hirst, 1920. Trans. Roy. Soc. Trop. Med. Hyg. 47:7.

Lee, R. D.: 1955a. The morphology of the Mexican chicken bug, *Haematosiphon inodorus* (Duges) (Hemiptera:Cimicidae). Pan-Pacific Entom. 31:47.

———: 1955b. New locality records and a new host record for *Haematosiphon inodorus* (Hemiptera.Cimicidae). Pan-Pacific Entom. 31:137.

Le Roux, A. C.: 1956. The safe, economical and practical destruction of *Argas persicus*, fowl tick, tampan, blue "bug," chicken tick or adobe tick. World's Poultry Sci. Jour. 12:285.

Lebouyries, G.: 1941. La Pathologie des Oiseaux. Vigot Frères, Paris, p. 816.

Levi, W. M.: 1957. The Pigeon. 2nd ed. Levi Publ. Co. Sumter, South Carolina. 667 pp.

Lindquist, W. D. and Belding, R. C.: 1949. A report on the subcutaneous or flesh mite of chickens. Mich. State Coll. Vet. 10:21.

Linduska, J. P., Morton, F. A., and McDuffie, W. C.: 1948. Tests of materials for the control of chiggers on the ground. Jour. Econ. Entom. 41:43.

Linkfield, R. L., and Reid, W. M.: 1958. Newer acaricides and insecticides in the control of ectoparasites of poultry. Jour. Econ. Entom. 51:188.

Loomis, E. C.: 1961. Life histories of ticks under laboratory conditions (Acarina: Ixodidae and Argasidae). Jour. Parasit. 47.91.

Loomis, R. B.: 1956. The chigger mites of Kansas (Acarina: Trombiculidae) Univ. Kansas Science Bul. 37 (part II):1195.

MacCreary, D., and Catts, E. P.: 1954. Ectoparasites of Delaware poultry including a study of litter fauna. Univ. Del. Agr. Exper. Sta., Tech. Bul. 507. 22 pp.

Madden, A. H., Lindquist, A. W., and Knipling, E. F.: 1944. Tests of repellents against chiggers. Jour. Econ. Entom. 37.283.

———, Lindquist, A. W. and Knipling, E. F.: 1945. DDT and other insecticides as residual type treatments to kill bedbugs. Jour. Econ. Entom. 38:265

Mandoul, R., Tempere, G., and Newil, J.: 1945. *L'acariase dermanyssique humaine. Soc. Hist. Nat, Bul.* (Toulouse) 80:199.

Marcovitch, S.: 1926 The control of poultry lice and mites (Sodium fluosilicate). Tenn. Agr. Exper. Sta., Circ. 2.

Martin, M.: 1934 Life history and habits of the pigeon louse *Columbicola columbae* (Linn.). Canad. Entom. 66 6.

Matheson, R., Brunett, E. L., and Brody, A. L.: 1931. The transmission of fowl pox by mosquitoes. Preliminary report. Poultry Sci. 10:211.

Maw, W. A., Whitehead, W. E., and Bemont, L. H.: 1935. The northern fowl mite and its control. Scient. Agr. 16.79.

Mégnin, P.: 1879. Les acariens parasites du tissue cellulaire et des réservoirs aériens chez les oiseaux. Jour. Anat. et Physiol. 15:123

Melvin, R., Smith, C. L., Parish, H. E., and Barrett, W. L., Jr.: 1941. A new remedy for the prevention and treatment of screwworm infestation of livestock. Bur. Entom. and Plant Quaran. U.S.D.A., Publ. E-540 (mimeographed).

Menon, P. B., Sen Gupta, C., and Basu, B. C.: 1951. Studies on the feeding of insecticides for the control of ectoparasites. Indian Vet. Jour. 27:430.

Metcalf, R. L.: 1948. Organic Insecticides: Their Chemistry and Mode of Action. Interscience Press, New York

———: 1957. Advances in Pest Control Research. Vols. 1-5 (1957-1962). Interscience Publishers, New York.

Metz, K.: 1911 *Argas reflexus*, die Taubenzecke Monatschr. f. prakt. Tierchelk. 22:481.

Meyer, K. F., and Eddie, B.: 1960. Feather mites and ornithosis. Science 132:300.

Michener, C. D.: 1946. Observations on the habits and life history of a chigger mite, *Eutrombicula batatas* (Acarina. Trombiculinae). Ann. Entom. Soc. Am. 39:101.

Micks, D. W.: 1951 The laboratory rearing of the common fowl tick, *Argas persicus* (Oken). Jour. Parasit. 37.102.

Miles, V. I., Howitt, B. F., Gorrie, R., and Cockburn, T. A.: 1951. Encephalitis in Midwest. V. Western equine encephalitis virus recovered from mites, *Dermanyssus americanus* Ewing. Proc. Soc. Exper. Biol. and Med. 77:395.

Moffatt, B. W.: 1955. The stickfast flea of poultry. Queensland Agr. Jour. 81:239.

Moore, S., III: 1952. Control and eradication of chicken lice with lindane. Poultry Sci. 31:444.

———, and Schwardt, H. H.: 1954. The control of external parasites of chickens in New York State. Poultry Sci. 33:1230.

Myers, L. E.: 1928. The American swallow bug, *Oeciacus vicarus* Horvath. Parasit. 20.159.

Najera, L., and Mayer, H. F.: 1951 Action of some insecticides on *Argas persicus.* (Trans. title) Rev. Ibérica Parasit. 11:61.

Neveu-Lemaire, M.: 1938. Traité d'Entomologie Médicale et Vétérinaire. Vigot Frères, Paris.

Nuttall, G. H. F., and Warburton, C.: 1908. Ticks, a Monograph of the Ixodidae. Part I, Argasidae: Part II, Ixodidae. Cambridge (England) Univ. Press.

Olson, C.: 1935. The effect of certain ectoparasites on the cellular elements and hemoglobin of the blood of the domestic chicken. Jour. Am. Vet. Med. Assn. 87:559.

O'Roke, E. C.: 1930. The morphology, transmission, and life history of *Haemoproteus lophortyx* O'Roke, a blood parasite of the California valley quail. Univ. of Calif. Publ. in Zool. 36:1.

———: 1934. A malaria-like disease of ducks, caused by *Leucocytozoon anatis* Wickware. Univ. of Mich, School of Forestry and Conserv., Bul. 4.

Parish, H. E.: 1942. Phenothiazine protects chickens from lice in Texas test. E. I. du Pont de Nemours and Co., Agr. News Letter 10:35.

———: 1943. Sodium fluosilicate to control poultry lice. Jour. Econ. Entom. 36:353.

Parker, W. L., and Beacher, J. H.: 1947. Toxaphene vs. DDT for bedbugs and roaches. Del. Agr. Exper. Sta., Bul. 264. 17 pp.

Parman, D. C.: 1923. Biological notes on the hen flea, *Echidnophaga gallinacea*. Jour. Agr. Res 23:1007.

———, Abbott, W. S., Culver, J. J., and Davidson, W. M.: 1928. Ineffectiveness of internal medication of poultry for the control of external parasites. U.S.D.A., Tech. Bul. 60.

Patton, J. W.: 1931. The fowl tick, *Argas miniatus*. Poultry Sci. 1:125.

Payne, L. F.: 1929. A new method of controlling feather mites. Jour. Econ. Entom. 22:819.

———: 1930. Feather mites and their control. Ala. Polytech. Inst., Bul. Vol. 25 (1) pp. 61–63 (1930). Twenty-first Ann. Meeting, Proc. of Poultry Sci. Assn.

Peterson, R. H.: 1949. Field tests of some insecticides in the control of the common red mite of poultry and of the northern fowl mite. Poultry Sci. 28:411.

Pillers, A. W. N.: 1921. Notes on mange, and allied mites for veterinarians. Baillière, Tindall, and Cox. London.

———: 1927. Perforations in pigeons' feathers due to the mite, *Falculifer rostratus* (Buchholz). Vet, Jour. 83:410.

Pinto, G.: 1930. Arthropodos Parasitos e Transmissores de Doenças. Mello e C., Rio de Janeiro

Plasaj, S.: 1925. Sur la transmission du choléra aviaire par les *Dermanyssus*. Jugoslav. Vet. Glasnik., Livr. 1–6. (*Rev. in Rev. Gén. de Méd. Vét. 34:654*)

Povar, M. L.: 1946. Value of DDT for the control of the northern feather mite, *Liponyssus sylviarum*. Cornell Vet. 36:51.

Prouty, M. J., and Coatney, G. R.: 1934. Further studies on the biology of the pigeon fly, *Pseudolynchia maura* Bigot. Parasit. 26,219

Quigley, G. D., and Cory, E. N.: 1946. The utility of DDT for the control of poultry ectoparasites. Poultry Sci. 25:419.

Raun, E. S.: 1956. Chicken louse and mite control with malathion formulations. Jour. Econ. Entom. 49,628

———, and Nelson, C. L.: 1956. How to delouse 4000 toms in 100 minutes. Turkey World 31: (May) 12.

Readio, P. A.: 1927. Studies on the biology of the Reduviidae of America north of Mexico, Univ of Kans., Sci. Bul. 17.5.

Rebrassier, R. E. and Martin, E. D.: 1932. *Syringophilus bipectinatus*, a quill mite of poultry. Science 76:128.

Reeves, W. C., Hammon, W. McD, Doetschman, W. H., McClure, H. E., and Sather, G.: 1955. *Studies on mites as vectors of western equine and St. Louis encephalitis viruses in California.* Am Jour. Trop. Med. and Hyg. 4,90.

———, Hammon, W. M., Furman, D. P., McClure, H. E. and Brookman, B.: 1947. Recovery of western equine encephalomyelitis virus from wild bird mites (*Liponyssus sylviarum*) in Kern County, California. Science 105:411.

Reid, W. M., and Ackert, J. E.: 1937. The cysticercoid of *Choanotaenia infundibulum* (Bloch) and the housefly as its host. Trans. Am. Micro. Soc. 56.99

———, and Linkfield, R. L.: 1957. New distribution record and economic importance of *Menacanthus cornutus* (Schommer) on Georgia broilers. Jour. Econ. Entom. 50:375.

———, Linkfield, R. L., and Lewis, G.: 1956. Limitations of malathion in northern fowl mite and louse control. Poultry Sci. 35:1397.

Reis, J.: 1939. Alguns parasitas de *Gallus gallus* (*L*) verificados em São Paulo. Arq. Inst. Biol 10:147.

———, and Nobrega, P.: 1956. Tratado de Doenças das Aves. 2nd ed. Comp. Melhoramentos de São Paulo. Industrias de Papel, Caixa Postal 8120, São Paulo, Brazil. 4 vols. in 2 parts.

Reizr, L. P.: 1960. Biological and Chemical Control of Plant and Animal Pests. Amer. Assoc. Adv. Sci. Washington, D.C. 256 pp.

Richardson, H. H.: 1943. Studies of methyl bromide, chloropicrin, certain nitriles and other fumigants against the bedbug. Jour. Econ. Entom. 36:420.

Richter, P. O., and Inako, W. M. Jr.: 1948a. External parasites of chickens and their control. Ky. Agr. Exper. Sta., Bul. 517. 25 pp.

———, and Inako, W. M., Jr. 1948b. Control of the northern fowl mite, *Liponyssus sylviarum* (C. and F.) Jour. Econ. Entom. 41:325.

Roberts, F. H. S., O'Sullivan, P. J., Rumball, P., and McLauchlan, A. W.: 1947. Observations on the value of DDT for the control of the poultry stickfast flea, *Echidnophaga gallinacea* Westwood. Australian Vet. Jour. 23:168.

Roberts, I. H., and Peterson, H. O.: 1947. Hexachlorocyclohexane—a fumigant for the control of chicken lice. Poultry Sci. 26 588.

———, and Smith, C. L.: 1956a. Poultry lice. *In:* Animal Diseases. U.S.D.A. Yearbook 490.

———, and Smith, C. L.: 1956b. Mites on poultry. *In:* Animal Diseases. U.S.D.A. Yearbook. 493.

Rodriguez, J. L., Jr., and Riehl, L. A.: 1956. Four pesticides tested against the fowl tick infesting turkeys in feed lots. Jour. Econ. Entom. 49:713.

———, and Riehl, L. A.: 1957a. Control of the chicken body louse on hens by self-treatment with malathion dust. Jour. Econ. Entom. 50:64.

———, and Riehl, L. A.: 1957b. Malathion spray for fowl tick control. Jour. Econ. Entom. 50:41.

———, and Riehl, L. A.: 1958. Malathion for control of chicken mites on hens in wire cages. Jour. Econ. Entom. 51:158.

———, and Riehl, L. A.: 1959. Spot treatments with malathion dust for control of the northern fowl mite on hens in individual wire cages. Jour. Econ. Entom. 52:13.

———, and Riehl, L. A.: 1960a. The malathion dust-bath for control of five species of lice on chickens. Jour. Econ. Entom. 53:328.

———, and Riehl, L. A.: 1960b. Malathion dust for chicken mite control. Jour. Econ. Entom. 53:328.

———, and Riehl, L. A.: 1960c. Control of northern fowl mite in community wire cages with malathion in special dust-bath boxes. Jour. Econ. Entom. 53:701.

———, and Riehl, L. A.: 1961. Suckfight flea control on chickens with malathion dust self-treatment. Jour. Econ. Entom. 54:1212.

———, and Riehl, L. A.: 1962. Control of flies in manure of chickens and rabbits by cockerels in Southern California. Jour. Econ. Entom. 55:473.

Rokey, N. W., and Snell, V. N.: 1951. Avian spirochetosis (*Borrelia anserina*) epizootics in Arizona poultry. Jour. Am. Vet. Med. Assn. 138:648.

Romaña, C., and Abalos, J. W.: 1948. Acción del "gammexane" sobre los triatomideos. "Control" domiciliario. Ann. d. Inst Med Regional; Uni. Nat. de Tucumán (Argentina). 2:95.

Schalk, A. F.: 1928. Results of some avian tuberculosis studies. (Transmission by fly larvae.) Jour. Am. Vet. Med. Assn. 72:852.

Schwabe, O.: 1956. A quill mite of poultry, a case report. (*Syringophilus bipectinatus*). Jour. Am. Vet. Med. Assn. 129:481.

Shaw, F. R., and Clark, G.: 1955. Notes on certain ectoparasites in Massachusetts. Jour. Econ. Entom. 46:1093.

Shepard, H. H.: 1958 and 1960. Methods of Testing Chemicals on Insects, Vol. 1. Burgess Publ. Co., Minneapolis, Minn. 355 pp; Vol. II. 248 pp.

Sherman, M., and Ross, E.: 1961. Toxicity to house fly larvae of droppings from chicks administered insecticides in feed, water, and as single oral dosages. Jour. Econ. Entom. 54:573.

Sikes, R. K., and Chamberlain, R. W.: 1954. Laboratory observations on three species of bird mites Jour Parasit. 40 691.

Simco, J. S., McPherson, B. N., and Lancaster, J. L., Jr.: 1962a. Controlling the chicken body louse. Arkansas Farm Research 11:7.

———, McPherson, B. N., and Lancaster, J. L., Jr.: 1962b. Control of the northern fowl mite. Arkansas Farm Research 11:10.

Skidmore, L. V.: 1932a. *Leucocytozoon smithi* infection in turkeys and its transmission by *Simulium occidentale* Townsend. Zentralbl. f. Bakt. I. Orig. 125:329.

———. 1932b. The transmission of fowl cholera to turkeys by the common house fly (*Musca domestica* Linn.) (with brief notes on the viability of fowl cholera microorganisms). Cornell Vet. 22:281

Smith, C L.: 1952. Field tests of insecticides against ectoparasites of poultry. Jour. Econ. Entom. 45.748.

Smith, C N.: 1951. Compounds more toxic to fleas than DDT. Am. Jour. Trop. Med. 31:252.

———, and Gouck, H. K.: 1944. DDT, sulfur and other insecticides for the control of chiggers. Jour Econ Entom 37:131.

———, and Gouck, H. K.: 1947. The control of chiggers in woodland plots. Jour. Econ. Entom. 40 790.

Smith, M. G., Blattner, R. J., and Heys, F. M.: 1944. The isolation of the St. Louis encephalitis virus from chicken mites (*Dermanyssus gallinae*) in nature. Science 100.362.

———, Blattner, R. J., and Heys, F. M.: 1945. Further isolation of St. Louis encephalitis virus; congenital transfer of virus in chicken mites (*Dermanyssus gallinae*). Proc. Soc. Exper. Biol. and Med. 59.136.

———, Blattner, R. J., and Heys, F. M.: 1946. St. Louis encephalitis: Infection of chicken mites, *Dermanyssus gallinae*, by feeding on chickens with viremia; transovarian passage of virus into the second generation. Jour. Exper Med. 84:1.

———, Blattner, R. J., and Heys, F. M.: 1947. St. Louis encephalitis: Transmission of virus to chickens by infected mites, *Dermanyssus gallinae*, and resulting viremia as source of virus for infection of mites. Jour. Exper. Med. 86:229.

———, Blattner, R. J., Heys, F. M., and Miller, A.: 1948. Experiments on the role of the chicken mite, *Dermanyssus gallinae*, and the mosquito in the epidemiology of St. Louis encephalitis. Jour. Exper. Med. 87:119.

Smyth, H. F., Jr.: 1956. The literature of pesticide toxicology. Jour. Agr. and Food Chem. 4.644.

Snyder, F. M., and Morton, F. A.: 1947. Benzyl benzoate-dibutyl phthalate mixture against chiggers. Jour. Econ. Entom. 40.586.

Solovcichik, L. L.: 1962. *Infestation of poultry with the itch mite (Laminosioptes cysticola)* (translated title). Veterinariya (Russia). 4:50.

Stagg, H. E.: 1946. The use of DDT in controlling fleas. Bur. Entom. and Plant Quaran., U.S.D.A., Rep. E-680.

Stenram, H.: 1956. The ecology of *Columbicola columbae* L. (Mallophaga). Opuscula Entom. (Sweden). 21:170.

Stewart, M. A.: 1927. A means of control of the European hen flea, *Ceratophyllus gallinae* Schrank. Jour. Econ. Entom. 20:132.

———: 1929. A case of cloacal myiasis in a hen and its treatment. Cornell Vet. 19:49.

———: 1932. Dispersal of the sticktight flea of hens (*Echidnophaga gallinacea* Westw.). Jour. Econ Entom. 25:164

Stockdale, H. J., and Raun, E. S.: 1960. Economic importance of the chicken body louse. Jour. Econ. Entom. 53 421.

Sulkin, S. E.: 1945. Recovery of *equine encephalomyelitis virus (western type) from chicken mites*. Science 101:381.

———, and Izumi, E. M.: 1947. Isolation of western equine encephalomyelitis virus from tropical fowl mites, *Liponyssus bursa* (Berlese). Soc. Exper. Biol. and Med Proc. 66:249.

———, Wisseman, C. L., Jr., Izumi, E. M., and Zarafonetis, C.: 1955. Mites as possible vectors or reservoirs of equine encephalitis in Texas. Am. Jour. Trop. Med, and Hyg. 4:119.

Sullivan, K. C.: 1924. The use of calcium cyanide for the control of fleas and chicken lice. Jour. Econ. Entom. 17:250

Telford, H. S.: 1945a. New insecticides for chicken lice control. Jour. Econ. Entom. 38:573.

———: 1945b. DDT as a chicken louse control. Jour. Econ. Entom. 38:700.

———: 1947. Benzene hexachloride to control certain insects affecting domestic animals. Jour. Econ. Entom. 40:918.

———, and Guthrie, J. E.: 1946. Use of DDT in livestock sprays. Agr. Chem. I (No. 3):29 and 84.

Theodorides, J.: 1949. Les Coléoptères nuisibles aux animaux domestiques, Ann. Parasit. Hum. et Comp. 24:116.

Thompson, R. P., and Hosking, W. F.: 1957. A rount of Mallophaga on a heavily infested hen. Poultry Sci. 36.213.

Tower, D. A., and Floyd, E. H.: 1961a. Consumer and organoleptic tests with broilers grown on lindane-impregnated litter. Poultry Sci. 40:234.

———, and Floyd. E. H.: 1961b. The effect of the chicken body louse, *Eomenacanthus stramineus* on egg production in New Hampshire pullets. Poultry Sci. 40:393.

Underhill, G. W.: 1959. Two simuliids found feeding on turkeys in Virginia. Jour. Econ. Entom. 32:765.

———. 1944. Blackflies found feeding on turkeys in Virginia (*Simulium nigroparvum* Twinn and *S. slossonae* Dyar and Shannon). Va. Agr. Exper. Sta., Tech. Bul. 94.

U.S. Department of Agriculture: 1953. Bed bugs; how to control them. Leaflet 337, 8 pp.

———. 1953. Fleas: how to control them. Leaflet 392 (not paged).

———. 1956. Chiggers; how to fight them. Leaflet 403. (not paged).

———. 1962a. The fowl tick: how to control it. Leaflet 382. 2 pp.

———. 1962b. Poultry mites: how to control them. Leaflet 383. 7 pp.

———. 1962c. Controlling mosquitoes in your home and on your premises. Home and Garden Bul. 84, 12 pp.

Usinger, R. L.: 1947. Native hosts of the Mexican chicken bug, *Haematosiphon inodora* (Dugés) (Hemiptera, Cimicidae). Pan-Pacif. Entom. 23:140.

van Heckinergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. F. Enke, Stuttgart.

Vincent, L. E., Lindgren, D. L., and Krohne, H. E.: 1954. Toxicity of malathion to the northern fowl mite. Jour. Econ. Entom. 47:943.

Walker, G. P.: 1927. A blackfly (*Simulium bracteatum*) fatal to goslings. Canad. Entom. 59:123.

Ware, G. W.: 1961. BHC contamination of chicken eggs from treated litter. Jour. Econ. Entom. 54:802.

———, and Naber, E. C.: 1961. Lindane in eggs and chicken tissues. Jour. Econ. Entom. 54:675.

Warren, D. C.: 1945. The value of DDT for the control of the common chicken louse. Poultry Sci. 24:473.

———, Eaton, R., and Smith, H.: 1948. Influence of infestations of body lice on egg production in the hen. Poultry Sci. 27.641.

Wells, R. W., Bishopp, F. C., and Laake, E. W.: 1922. Derris as a promising insecticide. Jour. Econ. Entom. 15:90.

West, T. F., and Campbell, G. A.: 1946. DDT, the Synthetic Insecticide. Chapman and Hall, London. 301 pp.

Wharton, G. W., and Fuller, H. S.: 1952. A Manual of the Chiggers. Mem. Entom. Soc. Wash., No. 4. 185 pp.

Whitehead, W. E.: 1942. Lice and some other external parasites of domestic animals and poultry in the province of Quebec. Macdonald College, McGill Univ., Farm Bul. 7.

Whitlock, J. H.: 1960. Diagnosis of Veterinary Parasitisms. Lea and Febiger Co., Philadelphia, Pa. 236 pp.

Wickware, A. B.: 1921. An unusual form of scabies in fowls. Jour. Parasit. 8:90. *(Megninia gallinulae).*

Wilkins, S. D., and Dutcher, R. A.: 1920. Limberneck in poultry. (Fly larvae.) Jour. Am. Vet. Med. Assn. 57.653.

Wilson, F. H.: 1933. A louse feeding on the blood of its host. Science 77:490. *(Menopon stramineum.)*

———: 1934. The life-cycle and bionomics of *Lipeurus heterographus* Nitzsch. Jour. Parasit. 20.304.

———: 1939. The life-cycle and bionomics of *Lipeurus caponis* (Linn). Entom. Soc. Am. Ann. 32.318.

———: 1941. The slender lice of American pigeons and doves, with descriptions of two new species. Jour. Parasit. 27:259.

Wilson, H. G., and Gahan, J. B.: 1957. Control of house fly larvae in poultry houses. Jour. Econ. Entom. 50.613

Wisseman, C. L., Jr., and Sulkin, S. E.: 1947. Observations of the laboratory care, life cycle, and hosts of the chicken mite, *Dermanyssus gallinae.* Am. Jour. Trop. Med. 27:463.

Wolford, J. H., Ringer, R. K., Coleman, T., H., and Zindel, H. C.: 1962. Nicotine sulfate treatment of turkey breeder hens housed in individual cages. Quart. Bul. Mich. Agr. Exp. Sta. 44:759.

Wright, W. H.: 1944. The bedbug, its habits and life history and methods of control. U.S. Public Health Service, Public Health Rep., Suppl. 175. 9 pp.

Yager, R. H., and Gleiser, C. A.: 1946. *Trichomonas* and *Haemoproteus* infections and the experimental use of DDT in the control of ectoparasites in a flock of Signal Corps pigeons in the Territory of Hawaii. Jour. Am. Vet. Med. Assn. 109.204.

Yates, W. W.: 1953. Notes on the ecology of *Culiseta* mosquitoes found in the Pacific Northwest. Mosquito News 13 229.

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34

Nematodes and Acanthocephalids of Poultry

Nematodes

Nematodes or roundworms are usually elongated, cylindrical, and unsegmented worms. The body is covered with a tough, noncellular layer known as the cuticle. These worms have a well-developed alimentary tract and, in contrast to the tapeworms, are usually bisexual.

The class Nematoda is divided into a number of orders, the members of which are parasitic, semiparasitic, and free-living. In the present article, only those forms that are parasitic in poultry are discussed.

The following key will aid in differentiating the eight families containing species of poultry-parasitic nematodes.

1. Worms with free-living adult generation, that is, males and females developing outside of body; in digestive tract, hermaphroditic females only . . . . Strongyloididae
   Worms without a free-living generation, that is, incapable of producing males and females outside of body 2
2. Worms hairlike or threadlike; esophagus tubular and capillary, the tube embedded in or otherwise in relation to a single row of cells; in crop and small intestine . . . . Trichuridae
   Worms thick as compared with above; esophagus well developed and muscular and with definite triangular lumen, not in relation to a single row of cells 3
3. Cordons or other cephalic ornamentations present . . . . Acuariidae
   Cordons or other cephalic ornamentations absent 4
4. Preanal sucker present 5
   Preanal sucker absent 6

5. Esophagus with distinct posterior bulb containing a valvular
   apparatus . . . .   Heterakidae
   Esophagus without a distinct posterior bulb . . . .   Ascarididae
6. Bursa present   7
   Bursa absent   8
7. Buccal capsule well developed and containing at least six teeth at base;
   oral opening hexangular . . . .   Syngamidae
   Buccal capsule reduced and containing not more than three teeth at base
   or none . . . .   Trichostrongylidae
8. Pseudolabia absent . . . .   Thelaziidae
   Pseudolabia present . . . .   Spiruridae

General Morphology. In general the body of a nematode is spindle-shaped with the anterior and posterior ends attenuated. The body covering or cuticle is usually marked by transverse grooves, and sometimes longitudinal folds or alae may be present. These alae may be confined to the anterior end of the body, in which case they are termed cervical alae; or they may be confined to the posterior part of the body, being then termed caudal alae. The latter are found on the tail of the male worm and, in the case of certain groups, are modified to form a bursa. Cuticular ornamentations are occasionally found on the anterior extremities of certain small groups of roundworms. These ornamentations may take the form of spines, cordons, or shields.

The mouth opening is located at the extreme tip of the anterior end of the body and is usually surrounded by lips bearing sensory organs. In the more generalized type of nematodes, the mouth leads directly into a mouth cavity. This cavity may be considerably reduced or absent in the more specialized groups of nematodes. Directly posterior to the mouth cavity is the esophagus. This part of the intestinal tract may be simple, i.e., consisting of one undivided part, or it may be more complex, consisting of a short, anterior muscular part and a long posterior glandular part. A bulb may or may not be present at the posterior end of the esophagus. Following the esophagus is the intestine which is connected with the anal or cloacal opening in the posterior end of the body by a short rectum.

The nematodes are, with very few ex-

ceptions, sexually distinct. The male can usually be distinguished from the female by the presence of two—sometimes only one—chitinous structures known as spicules which are located in the posterior end of the body. The spicules have been considered as intromittent organs for use during copulation. That the spicules do take an active part in copulation has been observed many times. They have been observed to withdraw and insert alternately over extended periods during coitus. It has been reported that the primary functions of the spicules is to keep the vulva and vagina open and, to some extent, guide the sperm into the female. The female reproductive products are discharged through the vulva, the position of which varies considerably in different groups of nematodes.

Sexual dimorphism is remarkably demonstrated by some species of nematodes. One of the most striking examples of this interesting phenomenon is *Tetrameres americana*, a nematode occurring in the proventriculus of certain kinds of poultry. The globular-shaped females enter the glands of the proventriculus or glandular stomach when very young, and as they begin to swell with eggs, their bodies assume the shape of the lumen of the glands. The adult male worm of this species is very much smaller than the female and retains the usual elongated nematode shape and usually lives free in the lumen of the proventriculus.

Nematodes are found in a variety of locations within the bodies of their hosts. The eyes, air sacs, thoracic and abdominal cavities, and the tracheae are some of the

unusual places that nematodes occur as parasites of avian hosts. The intestinal tract is, of course, the habitat of the largest number of species of roundworms.

Development. Nematodes have both a direct (monoxenous) and an indirect (heteroxenous) type of development. Those worms with life histories of the first type require no invertebrate intermediate hosts to complete their life cycles and constitute approximately one-third of all the nematode species infecting poultry. However, the majority of the species of roundworms found in poultry are of the second type and depend upon such intermediate hosts as insects, snails, and slugs for the early stages of their development.

Regardless of the type of development that a certain species of nematode may have, it normally passes through four developmental stages before it becomes an adult; the latter is the final or fifth stage. Beginning with the second stage, each succeeding developmental stage in the life cycle is preceded by a molt. A molt is usually referred to as a shedding of the skin. In the case of some nematodes the loosened skin or cuticle may sometimes be retained for a short time as a protective covering, while in others it is shed at once.

Aside from the fact that certain nematodes require intermediate hosts to complete their development and others do not, the life histories of most nematodes infecting poultry are essentially the same. The eggs, which are deposited in the location in which the female worms are found, ultimately reach the outside in the droppings. This exo-corporal existence is apparently essential in order that the eggs may be rendered infective for the next host, be it avian or arthropod. The conditions existing within the body of the definitive host are usually inimical to the development of the eggs. However, when once outside the body of the bird host and in the presence of optimum moisture and temperature requirements, these eggs undergo development. The time required for the eggs to embryonate depends somewhat upon the species of parasite since,

under similar environmental conditions, the eggs of some nematodes require only a few days to complete embryonation, while others require several weeks. In the case of nematodes with a direct life cycle, the final host becomes infected by eating the embryonated eggs or the freed larvae. On the other hand, in the case of nematodes with an indirect life cycle, the intermediate host ingests the embryonated eggs or free larvae and retains the larvae within its body tissues. When a suitable final host eats the infested intermediate host containing infective larvae, it will become infected.

Importance of nematodes of poultry. Nematodes, as a whole, constitute the most important group of helminth parasites of poultry. Both in number of species affecting poultry and in the amount of damage done, this group of parasites far exceeds the flukes and cestodes.

Some of our most important individual worm parasites of poultry are found in this group. One species, *Heterakis gallinarum*, plays an important role in the transmission of the protozoan disease known as blackhead. Although considered of little economic importance as parasites of the domestic fowl, this species apparently has caused serious and enormous losses to the poultry industry in the role of a carrier of the blackhead organisms. The gapeworm is perhaps the most serious nematode parasite of young poultry, particularly chickens and turkeys. Until recently, this worm was responsible for considerable losses among young birds both in this country and in Europe. Before changed poultry husbandry practices and other effective control measures reduced its devastating losses, this parasite was dreaded as much as blackhead. Despite all of our efforts to control this worm, serious outbreaks of epidemics among pheasants continue to be reported as due to the poultry gapeworm. Suffice to say, most of the nematodes parasitic in poultry and closely related birds may inflict serious injury to their respective hosts if infections are sufficiently large.

The following list shows species of nematodes found in poultry in this country, with their intermediate hosts, usual locations, and kinds of poultry affected.

NEMATODES OF THE EYE
Thelaziidae

The eyes of poultry are the seat of infection for a single species of nematode, *Oxyspirura mansoni*. This nematode belongs to the Thelaziidae. Members of this family have a buccal capsule which may be well developed or rudimentary, and the vulva may be anterior or posterior to middle of body.

Oxyspirura mansoni (Cobbold, 1879)

Synonym. *Filaria mansoni* Cobbold, 1879.

Description. Mouth circular, surrounded by a 6-lobed chitinous ring (Fig. 34.1A). Two pairs of subdorsal and 1 pair of subventral teeth in mouth cavity.

Male 8.2 mm. to 1.6 cm. long by 350μ wide. Tail curved ventrally, without alae.

Four pairs of preanal and 2 pairs of postanal papillae (Fig. 34.1B). Spicules unequal (Fig. 34.1C); one is 3 to 4.55 mm. long and the other 180 to 240μ long.

Female 1.2 to 2 cm. long by 270 to 430μ wide. Vulva 780μ to 1.55 mm. from tip of tail. Eggs embryonated when deposited.

This roundworm is found beneath the nictitating membrane, conjunctival sacs, and naso-lacrimal ducts of poultry in the states of Florida and Louisiana, and possibly other southern states.

Life history. Sanders (1929) found that an intermediate host was necessary for the successful transmission of this parasite from one bird host to another.

The complete life cycle of the eyeworm as worked out by Sanders is as follows: The eggs of the mature female worm are deposited in the eyes of the bird host. They are then washed down the tear ducts, swallowed, and passed to the exterior in the droppings. The cockroach, *Pycnoscelus (Leucophaea) surinamensis*, which is an
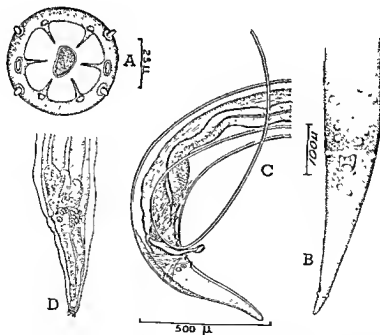
FIG. 34.1 — Oxyspirura mansoni. (A) Front view of head. (B) Ventral view, and (C) lateral view, of male tail. (After Ransom, 1904.) (D) Tail of second stage larva. (After Fielding, 1928.)

Nematodes	Location	Intermediate hosts	Definitive hosts
Oxyspirura mansoni	Eye	Cockroaches	Chicken, Turkey, Peafowl, Ducks
Syngamus trachea	Trachea	None	Chicken, Turkey, Guinea fowl, Goslings, Pheasant
Cyathostoma bronchialis	Trachea	Unknown	Geese
Capillaria annulata	Esophagus, Crop	Earthworms	Chicken, Turkey, Guinea fowl, Pheasant, Bobwhite quail
Capillaria contorta	Esophagus, Crop	None	Turkey, Duck, Bobwhite quail, Hungarian partridge, Ring necked pheasant
Gongylonema ingluvicola	Crop	Unknown	Chicken, Turkey, Bobwhite quail
Dispharynx nasuta	Proventriculus	Sowbugs Pillbugs	Guinea fowl, Turkey, Chicken, Pigeon, Bobwhite quail
Seurocyrnea colini	Proventriculus	Cockroaches	Turkey, Bobwhite quail, Sharp-tailed grouse, Prairie chicken
Tetrameres americana	Proventriculus	Grasshoppers Cockroaches	Chicken
Cheilospirura hamulosa	Gizzard	Grasshoppers Beetles Sandhoppers Weevils	Chicken, Turkey
Amidostomum anseris	Gizzard	None	Duck, Goose
Ascaridia galli	Small intestine	None	Chicken, Turkey
Ascaridia columbae	Small intestine	None	Pigeon
Ascaridia numidae	Small intestine	Unknown	Guinea fowl
Ascaridia dissimilis	Small intestine	None	Turkey
Capillaria obsignata	Small intestine	None	Pigeon, Chicken, Turkey
Capillaria caudinflata	Small intestine	Earthworms	Chicken, Turkey
Ornithostrongylus quadriradiatus	Small intestine	None	Pigeon, Mourning dove
Heterakis gallinarum	Cecum	None	Chicken, Turkey
Strongyloides avium	Cecum	None	Chicken, Turkey
Trichostrongylus tenuis	Cecum	None	Chicken, Duck, Goose, Guinea fowl
Subulura brumpti	Cecum	Grasshoppers Beetles Mealworms	Chicken, Turkey, Bobwhite quail
Subulura strongylina	Cecum	Unknown	Chicken, Guinea fowl, Bobwhite quail

omnivorous feeder, ingests the nematode eggs deposited in the droppings of infected birds. Within approximately 50 days following the ingestion of the infective eggs under experimental conditions, the cockroach contains in its body cavity mature larvae, which are capable of infecting a susceptible host. The mature larvae are often contained within cysts which are located deeply in the adipose tissue or along the course of the alimentary tract of the insect host. Some of the larvae release themselves from the capsules and are found free in the body cavity and legs of the cockroach. When an infected cockroach is swallowed by a chicken or other susceptible host, the infective larva is freed in the crop of the bird host, from which it later passes up the esophagus to the mouth and through the naso-lacrimal duct to the eye.

Experimental evidence indicates that various wild birds are capable of becoming infected with the eyeworm of poultry and, as a result, may serve as sources of infection for domestic birds. Such birds as the blackbird, *Agelaius phoeniceus*, the bobolink, *Dolichonyx oryzivorus*, the wild pigeon, *Columba livia*, the loggerhead shrike, *Lanius ludovicianus*, and the blue jay, *Aphelocoma cyanea*, have been experimentally infected with the eyeworm of poultry. Schwabe (1951) reported the eyeworm to occur naturally in the English sparrow, mynah, Chinese dove, Japanese quail, and pheasant (*Phasianus torquatus torquatus* and *P. versicolor versicolor*) in Hawaii. During the course of an investigation as to the role of the natural reservoir hosts in the spread of the eyeworm in Hawaii, Schwabe decided that the local wild birds are of little importance in the dissemination of this poultry parasite.

Pathology. Birds harboring eyeworms show a peculiar ophthalmia. They appear uneasy and continuously scratch at the eyes, which are usually watery and show much inflammation. The nictitating membrane becomes swollen and projects slightly beyond the eyelids at the corners of the eyes and is usually kept in continual motion as if trying to remove some foreign object from the eye. The eyelids sometimes become stuck together, and a white cheesy material collects beneath them. If left untreated, severe ophthalmia may develop, and as a result the eyeball may be destroyed. The worms are seldom, if ever, found in the eyes when severe symptoms are manifested, presumably due to unfavorable conditions existing there.

NEMATODES OF THE RESPIRATORY TRACT
Syngamidae

These roundworms, commonly designated as gapeworms, inhabit the trachea of young chickens, turkeys, and guinea fowls (Fig. 34.2). They are the cause of the disease known as "gapes." The gapeworm belongs to the family Syngamidae. Members of this family are characterized by having the vulva in the anterior part of the body, with stoma well developed and hexagonal in cross section, and with corona radiata reduced or absent.

Syngamus trachea (Montagu, 1811)

Synonyms. *Fasciola trachea* Montagu, 1811; *Syngamus trachealis* Siebold, 1836.

Description. Red worms, the color more pronounced in female. Mouth orbicular, with a hemispherical chitinous capsule, usually with 8 sharp teeth at the base. Mouth surrounded by a chitinous plate, the outer margin of which is incised to form 6 festoons opposite each other. Male permanently attached in copula to female, forming a Y (Fig. 34.3B).

Male 2 to 6 mm. long by 200μ wide. Bursa obliquely truncated, provided with rays, sometimes with strikingly asymmetrical dorsal rays. Spicules equal, slender, short, 57 to 64μ long.

Female 5 mm. to 2 cm. long (longer in the turkey) by 350μ wide. Tail end conical, bearing a pointed process. Vulva prominent, about one-fourth of body length from anterior end, but the position varies with age. Eggs 90μ long by 49μ wide, ellipsoidal, operculated (Fig. 34.3A).

The gapeworm, *Syngamus trachea*, is

FIG. 34.2 — Syngamus trachea. Trachea showing attached gapeworms.

FIG. 34.3 — Syngamus trachea. (A) Enlarged egg showing fully developed embryo. (B) Drawing of male and female gapeworms.
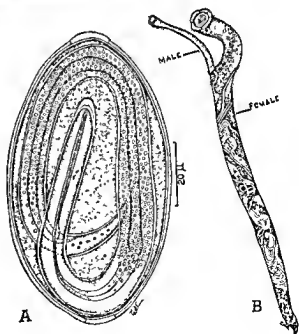
sometimes designated as the "red-worm" or "forked-worm" because of its red color and because the male and female are joined together so that they appear like the letter Y. This parasite is cosmopolitan in distribution.

Life history. The life history of the gapeworm is peculiar in that transmission of this parasite from bird host to bird host may be successfully accomplished either directly by the feeding of embryonated eggs or infective larvae, or indirectly by the ingestion of earthworms containing free or encysted gapeworm larvae which they had obtained by feeding on contaminated soil. The female gapeworm deposits its eggs through the vulvar opening underneath the bursa of the attached male into the lumen of the trachea. The eggs reach the mouth cavity, are swallowed, and pass to the outside in the droppings. Following a period of incubation of approximately 8 to 14 days under optimum conditions of moisture and temperature, these eggs become embryonated. Soon after embryonation, some of the eggs may hatch, the larvae living free in the soil. Should specimens of the earthworms, *Eusenia (H) foetidus* and *Allolobophora (H) caliginosus*, and perhaps others, be present in the soil which has been contaminated with feces containing the eggs of gapeworms, these annelids will become infected with gapeworm larvae. Within the earthworm, the larvae penetrate the intestinal wall, enter the body cavity, and finally invade the body musculature in which they may encyst for an indefinite period. Taylor (1938) stated that gapeworm larvae may remain infective to young chickens in the earthworm for as long as 4½ years. This author also found that slugs and snails may also serve as transfer hosts of *Syngamus trachea* larvae and that live gapeworm larvae were obtained from snails over a year after infection. These mollusks are not essentially true intermediate hosts in the strict sense of the word, since they are not absolutely necessary for the successful transfer of the gapeworm to other susceptible bird hosts.

Clapham (1934) and other investigators have observed that strains of *Syngamus trachea* taken from various wild and domestic birds were more readily transferred to young chickens and with a greater degree of success if the earthworm was employed as an intermediary.

The exact path of migration of the gapeworm larva after it has once entered the intestinal tract of the avian host until it reaches the lung is not known. Walker (1886) believed that the larvae, *after being swallowed by the definitive host, passed through the esophageal wall and entered the lungs directly from the outside*. More recent observations have indicated that the path of migration may be via the blood stream. However, convincing evidence which tends to show the true path of migration is still lacking.

Observations by Wehr (1937b) have shown that the infective larvae reach the lungs in an apparently unchanged condition within at least 6 hours after they have been ingested by the bird. By the third day following inoculation, the larvae have developed to the fourth stage, and by the seventh day several fourth-stage larvae and a few immature adults — one pair of the latter in copula — were found in the lungs; five pairs of immature adults in copula were found in the trachea. It is evident from these and other observations that the male and female of *Syngamus trachea* may copulate as young adults while in the lungs sometimes between the third and seventh days following infection, and that the worms reach the trachea about the seventh day after ingestion of the embryonated eggs and larvae. *These findings differ from those of Ortlepp (1923) who believed that the fourth stage was the final stage in the development of this nematode, and that the second-stage larvae represented the infective stage. This latter observation is obviously an error,* since the gapeworm embryo has been observed to molt twice inside the egg.

Approximately two weeks are required for the infective larvae to reach sexual maturity and for eggs to appear in the

droppings. About one-half of this time is spent in the lungs and the other half in the trachea.

The role played by the wild birds in the spread of gapeworm disease is still undecided. So far as it is known at the present time, wild birds probably are not an important factor in the spread of gapeworm disease in this country.

Pathology. Young birds are most seriously affected with gapeworms. The rapidly growing worms soon obstruct the lumen of the trachea and cause the birds to suffocate and die. Turkey poults, baby chicks, and pheasant chicks are very susceptible to infection with gapeworms, whereas the young of the other species of poultry which have been experimentally inoculated with the infective eggs and larvae of gapeworms are not so seriously affected. Turkey poults usually do not develop gapeworm symptoms earlier and begin to die sooner following gapeworm infection than young chickens. Experimentally infected guinea fowls, pigeons, and ducks do not exhibit characteristic symptoms of gapeworm infections. Young pheasants, however, suffer from the disease to an extent comparable to that of young chicks and turkey poults. Full-grown birds rarely show characteristic gapeworm symptoms, unless heavily infected. Investigators in this country have indicated that chickens 10 weeks or older are very difficult to infect experimentally with gapeworms. However, Crawford (1940) reported that gapeworms occurred commonly in the trachea of fowls of all ages, even in 3-year-old hens in Ceylon. He stated that the number of worms found in the trachea of each fowl was usually small and that adult hens not infrequently were seen to exhibit typical symptoms of gapeworm disease. He considered the adult fowl to be an important factor in the perpetuation of gapeworm disease in Ceylon. Olivier (1943) reported the occurrence of *Syngamus trachea* in mature chickens in Maryland. One of these birds was heavily infected and exhibited typical clinical symptoms.

Young birds infected with gapeworms show symptoms of weakness and emaciation, and usually spend much of their time with the eyes closed and the head drawn back against the body. From time to time they throw their heads forward and upward, and open the mouth wide in order to draw in air. It is not an uncommon occurrence to see an infected bird give its head a convulsive shake in an attempt to remove the obstruction from the trachea so that normal breathing may be resumed. Little or no food is taken by birds in the advanced stages of infection, and death is usually the end result.

An examination of the trachea of infected birds shows that the mucous membrane is extensively irritated and inflamed; coughing is apparently the result of this irritation to the mucous lining. Lesions are usually found to be present in the trachea of turkeys and pheasants, but seldom if ever seen in the trachea of young chickens and guinea fowls. Observations have shown that these lesions or nodules are produced as a result of an inflammatory reaction set up at the site of the attachment of the male worm. Since the attachment has been observed only at the lesions or point of attachment of the male worm and observations have shown that the head of the male is deeply embedded in the nodular tissue, it is, therefore, believed that the male worm usually remains permanently attached to the tracheal wall throughout the duration of its life. The female worms apparently detach and reattach from time to time in order to obtain a more abundant supply of food.

It must be remembered, however, that there are other diseases which may cause birds to gape, such as bronchitis and laryngotracheitis. In order that one may be sure just what is the cause of the gaping, it is necessary to make a postmortem examination of one or more of the affected birds. If gapeworms are not present in the trachea, bronchitis, laryngotracheitis or some other disease causing symptoms similar to gapeworm disease must be diagnosed.

Cyathostoma bronchialis (Muehlig, 1884)

Synonym. *Syngamus bronchialis* Muehlig, 1884.

Description. Very similar to *Syngamus*, but larger and less firmly united in copula. Buccal capsule somewhat wider than deep, usually six but occasionally seven triangular buccal teeth.

Male 8 to 12 mm. long by 200 to 600μ wide. Spicules long and slender, 540 to 700 mm. long, with tips slightly incurved.

Female 16 to 30 mm. long by 750μ to 1 5 mm. wide. Vulva with fairly prominent lips, situated in posterior part of anterior third of body. Tail acute. Eggs 68 to 90μ long and 43 to 60μ wide, with slight operculum in mature ones.

Life history. Unknown. Probably similar to *Syngamus trachea*. This species of gapeworm is apparently widespread in domestic geese in Europe and to some extent in wild geese of the United States. The following record of its appearance in domestic geese of this country appears to be the only one.

Pathology. Griffiths *et al.* (1954) reported a morbidity of 80 per cent with a mortality of about 20 per cent in a flock of domestic geese near Duluth, Minnesota. The course of the disease extended over a period of 5 months, during which time the birds showed symptoms of respiratory distress as evidenced by throwing back their heads and gaping for air. Severely affected birds died soon after the appearance of respiratory disturbances. The symptoms exhibited were similar to those of laryngotracheitis. Recovered birds showed growth retardation. Cram (1928) reported infections of this gapeworm in Young Blue, Cackling, Snow, and Canada geese in Illinois.

NEMATODES OF THE ALIMENTARY TRACT

CROP

At least three species of nematodes, commonly referred to as crop worms, occur in the crop of domestic fowls. Two of these are commonly known as capillarid worms or hairworms and belong to the family Trichuridae, while the third is designated as the gullet worm and is a member of the family Thelaziidae.

Trichuridae

The members of this family are characterized by having the body more or less clearly divided into an esophageal portion and a posterior portion which contains the other organs. The esophagus is a cuticular tube embedded in one side of a single or double row of esophageal glands. The male possesses only a single spicule. Vulva is located at junction of esophageal and posterior portions of body.

Capillaria annulata (Molin, 1858)

Synonyms. *Trichosoma annulatum* Molin, 1858; *Thominx annulata* (Molin, 1858) Cram, 1925.

Description. Worms long and threadlike. Cuticle inflated just behind head in adult specimens to form a bulbous swelling (Fig. 34.4A). Not far behind this, the cuticle is thrown into wavy transverse folds for a short distance.
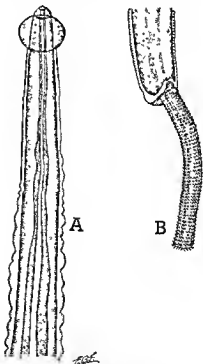
FIG. 34.4 — *Capillaria annulata.* (A) Head end. (B) Male tail. (After Cirurea, 1914.)

Male usually 1 to 2.5 cm. long by 52 to 74μ wide. Tail ends in two inconspicuous round lateral flaps, united dorsally by a cuticular flap. Spicule sheath beset with fine spines (Fig. 34.4B). Spicule 1.12 to 1.63 mm. long.

Female usually 2.5 to 6 cm. long by 77 to 120μ wide. Posterior portion of body (posterior to vulva) about 7 times as long as anterior portion. Vulva circular, located about opposite the termination of the esophagus. Eggs operculated, 55 to 66μ long by 26 to 28μ wide.

Capillaria annulata occurs naturally in the bobwhite quail, domestic chicken and turkey, pheasant, and Hungarian partridge. Worms of this species are similar in appearance to those of *Capillaria contorta* but may easily be differentiated by the presence of a cuticular swelling just back of the head.

Madsen (1951) synonymized *Capillaria annulata* (Molin, 1858) with *Capillaria contorta* (Creplin, 1839). Because of the presence of a cuticular inflation around the head and experimental evidence which points to the necessity of an intermediate host for its complete development, *Capillaria annulata* has been recognized as a distinct species.

Life history. Eggs of this parasite pass out in the droppings of the infected birds. They develop very slowly; a period of 24 days to over 1 month is sometimes necessary before they have reached the stage at which they contain an active embryo. Wehr (1936) discovered that the earthworm is required in order successfully to transmit *C. annulata* from one bird host to another. He demonstrated that under both natural and artificial conditions two species of earthworms, *Eisenia* (H) *foetidus* and *Allolobophora* (H) *caliginosus*, served as intermediate hosts of this crop worm. Chickens and other susceptible hosts become infected with this crop worm by swallowing infected earthworms.

Pathology. Cram (1926c) reported this worm as being associated with the deaths of turkeys in Maryland. The habit of burrowing into the crop mucosa causes a thickening of the crop wall and an enlargement of the glands in the areas in which the worms are located. Usually there is a slight or severe inflammation of the crop and esophageal walls, depending upon the severity of the infection. In heavy infections, the inner surface of the crop is immensely thickened, roughened, and badly macerated, the masses of worms concentrated primarily in this sloughing tissue (Fig. 34.5).

In pheasants, quail, and other gallinaceous game birds, infections with this

FIG. 34.5 — *Capillaria annulata*. Damage done to crop of quail, as compared with the thin-walled normal crop.

parasite often prove fatal. In these birds the symptoms reported are principally malnutrition and emaciation, associated with severe anemia. Allen and Gross (1926) reported severe anemia in an infected ruffed grouse, shortly before death.

Hung (1926) made a histopathological study of three cases of varying intensity and reported the following changes: "On the basis of the above observations it is quite evident that the pathological changes caused by *C. annulata* may be divided into three stages. The first stage is the hyperemic stage in which only hyperemia and lymphocytic infiltrations are present. In the second stage the yellowish-white nodules are present, and the lymphatic apparatus is enlarged and sometimes necrotic. *The enlargement of the lymph follicles gives the appearance of nodules* The third stage is that of the formation of the pseudomembrane, in which the mucosa is covered with a membrane containing fibrin."

Capillaria contorta (Creplin, 1839)

Synonyms. *Trichosoma contortum* Creplin, 1839, *Thominx contorta* (Creplin, 1839) Travassos, 1915.

Description. Body threadlike, attenuated anteriorly and posteriorly.

Male 8 mm. to 1.7 cm. long by 60 to 70μ wide Two terminal laterodorsal prominences on tail and Spicule very slender and transparent, about 800μ long, according to Travassos. Spicule sheath covered with fine hairlike processes (Fig. 34.6B).

Female 1.5 to 6 cm. long by 120 to 150μ wide. Vulva prominent, circular, 140 to 180μ posterior to beginning of intestine (Fig. 34.6A).

Capillaria contorta has been reported from a large number of hosts, including the duck, turkey (both domestic and wild), pheasant, quail, and ruffed grouse.

Life history. Eggs are apparently deposited in tunnels in the crop mucosa and escape into the lumen of crop and esophagus with the sloughed mucosa. They are found abundantly in droppings from infected birds. Approximately 1 month or slightly longer is required for embryos to develop within the eggs. Worms mature and eggs pass to the outside in the droppings of susceptible avian hosts from 1 to 2 months after feeding the embryonated eggs. Attempts to experimentally infect chickens, guinea fowls, and pigeons were unsuccessful.

Pathology. When present in large numbers these worms are highly dangerous. In light infections the wall of the crop and esophagus become slightly thickened and inflamed. In heavy infections, there is a marked thickening and inflammation, with a flocculent exudate covering the mucosa and with more or less sloughing of the mucosa (Fig. 34.7).

Affected birds become droopy, weak, and emaciated. Many deaths due to infection with this worm have been observed among wild turkeys and Hungarian partridges in this country.

The author visited a flock of domestic turkeys in Virginia, among which several birds were reported to have died from heavy infections with this crop worm. A number of visibly affected birds had been segregated from the main flock and were held in a pen by themselves. These birds moved only when disturbed, and then very slowly and with an unsteady gait. Occasionally a bird was seen to fall back on its hock joints and assume a penguin-like position. Others extended and retracted their heads and necks as if attempting to relieve an obstruction in their throats. The crops of the most severely affected birds were filled with a fetid liquid. Emmel (1939) observed that infection with this crop worm appeared first in the older birds, and later those of all ages became affected. Affected birds appeared indisposed, weak, and droopy, with the forepart of the body slightly elevated. The birds were not inclined to move unless forced to do so. He also observed that the birds occasionally assumed a penguinlike posture, with the head drawn close to the body, and that affected birds frequently swallowed and in doing so always extended and "ducked" their heads.
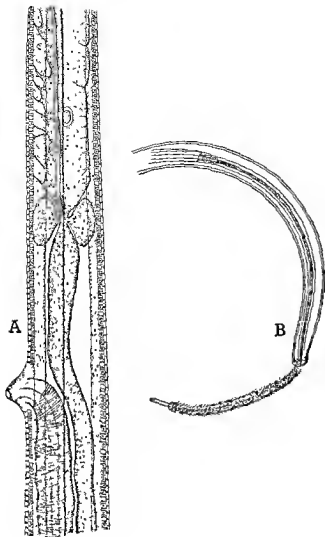
FIG. 34.6 — *Capillaria contorta*. (A) Region of vulva. (After Eberth, 1863.) (B) Male tail. (After Travassos, 1915.)

FIG. 34.7 — Section of crop of bobwhite quail, showing *Capillaria contorta* and damage produced by it. Experimental infection. ×114.

Thelaziidae

For family diagnosis, see page *969*.

Gongylonema ingluvicola Ransom, 1904

Description. Anterior end of body with a zone of shieldlike markings, few and scattered near head, numerous, and arranged in longitudinal rows farther back (Fig. 34.8A).

Male 1.7 to 2 cm. long by 224 to 250μ wide. Cervical papillae about 100μ from head end. Tail with two narrow bursal asymmetrical membranes. Genital papillae variable in number and asymmetrical; preanal papillae up to 7 on *left side* and up to 5 on the right side (Fig. 34.8B). Left spicule as long, or nearly as long (1.7 to 1.9 cm.) as body and 7 to 9μ wide, with a barbed point; right spicule 100 to 120μ long and 15 to 20μ wide.

Female 3.2 to 5.5 cm. long by 320 to 490μ wide. Vulva 2.5 to 3.5 mm. from tip of tail.

This worm has been reported from the chicken, turkey, and quail in the United States.

Life history. Cram (1931a) fed larval roundworms collected from the beetle,

FIG. 34.8—*Gongylonema ingluvicola.* (A) Head. (B) Male tail. (After Ransom, 1904.)

Copris minutus, to a chicken and recovered a single male specimen of a species of *Gongylonema,* tentatively identified as *G. ingluvicola.* Cram subsequently infected cockroaches by feeding embryonated eggs of *G. ingluvicola* derived from mountain quail. Some of the larvae recovered from the cockroaches were fed to a chicken. No worms were found when the chicken was killed 79 days later.

Pathology. The only damage that has been associated with these worms is the local lesions in the form of burrows in the mucosa of the crop.

General Discussion

Crop worms penetrate into the mucosa of the crop and esophagus and make tortuous burrows in which they live. Wehr (1937a) observed that each of these species of crop worms, when seen in their normal positions in the mucosa of the turkey, displayed a different body contour. The value of this observation lies in the fact that identifications of the worms involved may be made with a reasonable degree of accuracy without resorting to the time-consuming task of making a detailed microscopic examination of each worm. In situ, all three species of worms assume a folded position, differing, however, in the character of the folds. In the case of the so-called gullet worm, *Gongylonema ingluvicola,* the perspective is one of a series of folds approximately uniform in size and shape, following one another in close succession and usually extending in a straight line. The body shape of the two species of *Capillaria* consists of a series of irregularly shaped folds. *Capillaria annulata* may be differentiated from *Capillaria contorta* by its slightly smaller size. In case of any doubt as to the identity of the two species, the specimens may be removed from their burrows and examined under the microscope. Should a cuticular swelling be present directly back of the head, the worm is *C. annulata;* if this structure is absent, it is *C. contorta.*

Capillaria contorta is frequently found in large numbers in wild and domestic
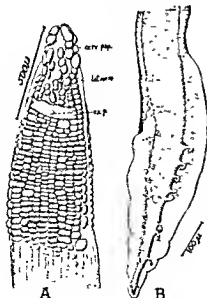
birds. In the United States, this crop worm has been reported to occur naturally in the Hungarian partridge, wild turkey, ring-necked pheasant, California valley quail, wild and domestic ducks, the domestic turkey, and possibly others.

STOMACH

Nematodes inhabiting the proventriculus of domestic poultry belong to two families, namely, Acuariidae and Spiruridae.

Acuariidae

The acuariids are characterized by having well-developed pseudolabia and cuticular ornamentations on the anterior part of the body.

Dispharynx nasuta (Rudolphi, 1819)

Synonyms, *Spiroptera nasuta* Rudolphi, 1819; *Dispharagus spiralis* Molin, 1858; *Acuaria spiralis* (Molin, 1858) Railliet, Henry, and Sisoff, 1912. Goble and Kutz (1945) concluded that all the forms of *Dispharynx* which have been recently recorded from galliform, columbiform, and passiform birds in the Western Hemisphere are nonspecific and that a morphological study of these forms leads them to consider their identity as *Dispharynx nasuta* (Rudolphi, 1819) Stiles and Hassall, 1920. *Dispharynx nasuta* (Rudolphi, 1819) Stiles and Hassall, 1920, has priority over *Dispharynx spiralis* (Molin, 1858) Skrjabin, 1916.

Description. Four wavy cuticular cordons on anterior end, originating at base of lips, recurrent, the distal extremity of the cordons turning forward and extending anteriorly a short distance (Fig. 34.9A). Postcervical papillae small, bicuspid, situated between the recurrent branches of the cordons. Body usually rolled in a spiral (Fig. 34.9B).

Male 7 to 8.3 mm. long by 250 to 315μ wide. Five pairs of postanal and 4 pairs of precanal papillae (Fig. 34.9C). Long spicule 400μ long, slender and curved; short spicule 150μ long, navicular.

Female 9 to 10.2 mm. long by 360 to 565μ wide. Small mucro on tip of tail.

Vulva in posterior portion of body. Eggs embryonated when oviposited.

This parasite has been encountered in the proventriculus of the chicken, turkey, guinea fowl, pigeon, pheasant, ruffed grouse, bobwhite quail, Hungarian partridge, and other gallinaceous birds. In addition, it has been found in a number of passerine birds in the United States. Yeatter (1934) found the incidence of this parasite among Hungarian partridge in the Great Lakes region to be 31.6 per cent. Bump (1935) stated that this worm was the most important parasite recovered from the ruffed grouse in New York State.

Life history. The pillbug, *Armadillidium vulgare*, and the sowbug, *Porcellio scaber*, were demonstrated to serve as intermediate hosts in experimental infections by Cram (1931b). The writer has repeatedly confirmed Cram's studies, using pigeons as definitive hosts. Within 4 days after the ingestion of the embryonated eggs by these isopods, the larvae have escaped from the eggs and are found among the tissues of the body cavity of the crustacean. The larva completes its development in the isopod within approximately 26 days; it has then reached the third or infective stage.

The definitive host becomes infected with the above nematode by swallowing infected pillbugs or sowbugs with the food or water. According to Cram (1931b) the female worms become sexually mature and are depositing eggs 27 days after ingestion by a susceptible vertebrate host.

Pathology. These roundworms are usually seen with their heads buried deeply into the mucosa. The formations of ulcers are often observed in the proventriculi of infected birds. In case of heavy infections, the wall of the proventriculus becomes tremendously thickened and macerated, tissue layers are indistinguishable, and the parasites become almost completely concealed beneath the proliferating tissue.

Allen (1925) believed that *D. nasuta* (*D. spiralis*) was the chief cause of "Grouse Disease" in northeastern United

States. Heavy infections of this parasite resulted in the death of many carrier pigeons of the Signal Corps of the United States Army, Fort Sam Houston, Texas, a number of years ago, according to Cram (1928). Several wild pigeons trapped at the Balboa Zoological Park, San Diego, California, and examined by the writer were found to be heavily infected with this parasite.

Seurocyrnea colini

Seurocyrnea colini is of common occurrence in the bobwhite quail of the southeastern states and has occasionally been collected from this same host in closely related birds in some of the northeastern states. It has also been reported from the turkey in Georgia, the prairie chicken in Wisconsin, and the sharp-tailed grouse in Wisconsin and Montana.

The preferred location of this nematode is in the wall of the proventriculus at its junction with the gizzard. The slender, yellowish-white worms are similar in appearance to *Cheilospirura hamulosa*, but are smaller and lack the so-called cordons or cuticular ornamentations on the an-
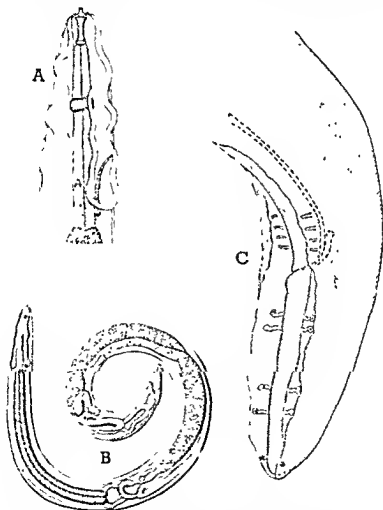
FIG. 34.9 → **Dispharynx nasuta.** (A) Head end. (After Seurat, 1915). (B) Female. Enlarged. (After Piana, 1897.) (C) Male tail. (After Cram, 1928.)

terior part of the body. The head structures are quite complicated, and the tail of the male has winglike expansions or alae (Fig. 34.10 A, B, C, D, and E).

The life history of this nematode is indirect, requiring the cockroach, *Blatella germanica*, as a temporary host. Since this intermediate host has been incriminated in an experimental role only, it is not known whether it actually serves in this same capacity under natural conditions.

There has been little or no pathological change observed in connection with infections of this parasite.

Spiruridae

The spirurids are characterized by having well-developed pseudolabia, cephalic papillae usually posterior to pseudolabia, and interlabia present or absent. The only
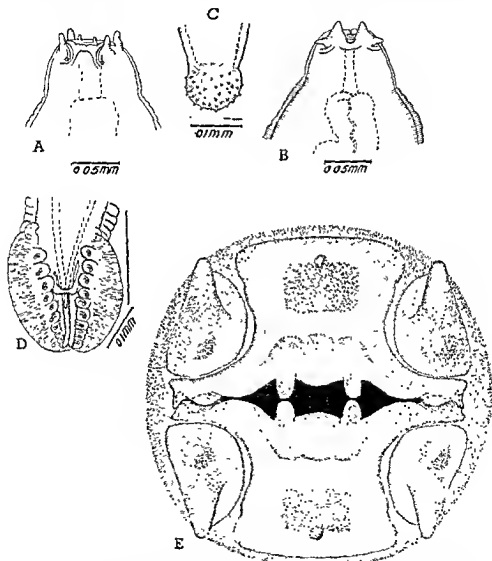
FIG. 34.10—*Seurocyrnea colini*. (A) Head, oblique lateral view. (B) Head, ventral view. (C) Tail of third-stage larva. (D) Male tail. (E) Head. En face view, *semidiagrammatic*.
[After Cram, 1927.]

member of this family found in poultry of this country have interlabia, and the sexes are distinctly dimorphic.

Tetrameres americana Cram, 1927

Description. Mouth surrounded with 3 small lips; buccal cavity present.

Male 5 to 5.5 mm. long by 116 to 133μ wide. Two double rows of posteriorly directed spines extend throughout whole body length, in the submedian lines. Cervical papillae present. Tail long and slender. Two unequal spicules, 100μ and 290 to 312μ long, respectively.

Female 3.5 to 4.5 mm. long by 3 mm. wide. Body globular, blood red in color, with 4 longitudinal furrows (Fig. 34.11). Uteri and ovaries very long, their numerous coils filling the body cavity.

The female of this species occurs in the glandular stomach of chickens and bobwhite quail (Fig. 34.12). At necropsy, these bright red worms are often observed through the wall of the unopened proventriculus. The male of this species is very small, almost microscopic in size, and resembles other nematodes in shape. It is very seldom observed elsewhere than on the surface of the mucosa of the proventriculus. However, the males of some of the species of *Tetrameres* occurring in wild birds have been found on several

occasions together with the females in the same glands. From all indications, it seemed that the two sexes, in the cases cited, were permanent residents of the glands in which they were found. When the male of *T. americana* enters the glands of the proventriculus, it apparently does so only long enough to mate with the female.

Cram (1931a) found this parasite to be common in quail which had been raised in captivity and in close proximity to poultry in Virginia. Stoddard (1931) reported *T. americana* as being found occasionally in quail raised in its natural habitat in the southeastern part of the United States.

Swales (1933) described *T. crami* from the proventriculus of a domestic duck in Canada. He stated that this species, of which the female only is known, differs from *T. americana* chiefly in the shorter muscular esophagus and the relative positions of the anus and vulva.

Another species of *Tetrameres*, *T. fissispina*, a species closely related to *T. americana*, has been reported from wild and domestic ducks and chickens in Europe. Sugimoto and Nishiyama (1937) stated that this roundworm was fairly common in chickens in Formosa.

Life history. Cram (1931b) discovered that *T. americana* required an intermediate host for its complete development. She fed embryonated eggs of this worm to two species of grasshoppers, *Melanoplus femurrubrum* and *M. differentialis*, and a species of cockroach, *Blatella germanica*, and recovered infective larvae from the body cavities of these insects in about 42 days after the ingestion of the eggs. When the grasshopper or cockroach is swallowed by a suitable bird host and digested in its stomach, the larvae escape; they remain in the proventriculus and develop into adults within a few days. The complete life cycle of *T. fissispina* involves such intermediate hosts as the amphipod, *Gammarus pulex*; the cladoceran, *Daphnia pulex*, and several species of grasshoppers, cockroaches, and earthworms.
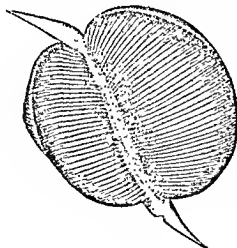
FIG. 34.11 — Tetrameres americana. Enlarged drawing of female. Original.
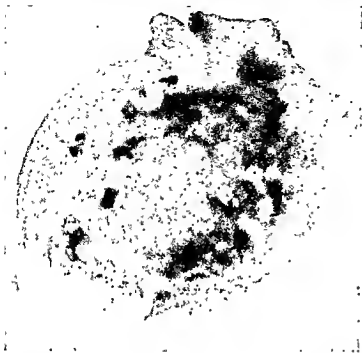
FIG. 34.12 — *Tetrameres americana.* Proventriculus showing female worms in glands. (After Cram, 1930.)

Pathology. According to Sugimoto and Nishiyama (1937), infected chickens become emaciated and anemic as a result of heavy infections. Cram (1931a) reported that *T. americana* has not been observed to produce any damage in quail. Barber (1916) stated that this proventricular worm was the cause of a serious catarrhal condition in chickens in Guam. In his report, he mentioned that the walls of the proventriculus were so thickened that the lumen was almost entirely obliterated; as many as 47 worms were found embedded in the wall.

GIZZARD

Gizzard nematodes belong to two families, namely, Acuariidae and Trichostrongylidae.

Acuariidae

For family diagnosis, see page 979.

Cheilospirura hamulosa (Diesing, 1851)

Synonyms. *Spiroptera hamulosa* Diesing, 1851.

Description. Two large, triangular, lateral lips. The 4 cuticular cordons double, irregularly wavy, and extending almost to

posterior extremity; not anastomosing or recurring anteriorly (Fig. 34.13A).

Male 9 mm. to 1.9 cm. long. Spicules very unequal and dissimilar, the left long and slender, the right short and curved. Tail tightly coiled; 2 very wide caudal alae present. Ten pairs of caudal papillae (Fig. 34.13B).

Female 1.6 to 2.5 cm. long. Vulva slightly posterior to middle of body. Tail pointed. Eggs embryonated when deposited.

This roundworm occurs commonly underneath the horny lining of the gizzard near the openings of the proventriculus and intestine in chickens and has occasionally been reported from the same locations in turkeys. It is widely distributed in the United States.

Life history. Investigations have shown that grasshoppers, beetles, weevils, and sandhoppers serve as intermediate hosts of *C. hamulosa* under natural as well as experimental conditions. Chickens and other susceptible avian hosts become infected with the adults of this roundworm by ingesting grasshoppers, beetles, weevils,

984   EVERETT E. WEHR
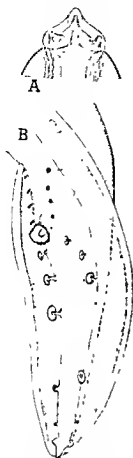
FIG. 34.13 — Cheilospirura hamulosa. (A) Head. (After Drasche, 1884.) (B) Male tail. (After Cram, 1931.)

and sandhoppers which are infected with larvae of this worm.

The infective or third stage larva may be recognized easily by the 2 prominent liplike structures at the anterior end of the body, the dorsal curvature of the posterior portion of the body, and the presence of four digitiform processes at the tip of the tail.

Pathology. When present in small numbers, these worms cause no evident effect on the health of the birds. In such infections, the lining of the gizzard may show small local lesions which may also involve the muscular tissue. Soft nodules enclosing parasites may be found in the muscular

portion of the gizzard. In heavy infections, the wall of the gizzard may be seriously damaged. Le Roux (1926) reported that this parasite may weaken the wall to such an extent as to cause it to rupture, with ultimate formation of a sac or pouch.

Trichostrongylidae

Members of this family are characterized by having reduced or rudimentary mouth cavity, corona radiata absent, and usually a well-developed bursa.

Amidostomum anseris (Zeder, 1800)

Synonyms. *Strongylus anseris* Zeder, 1800 in part; *Amidostomum nodulosum* (Rudolphi, 1803) Seurat, 1918.

Description. Worms slender and reddish. The short wide buccal capsule has 3 pointed teeth at its base (Fig. 31.14A).

Male 10 to 17 mm. long by 250 to 350μ wide. Bursa with 2 large lateral lobes and a small median lobe (Fig. 31.14C). Dorsal ray short, bifurcating posteriorly and the bifurcations forked and terminating in 2 tips. Spicules 200μ long, slender, and cleft near their middle. Gubernaculum slender and 95μ long.

Female 12 to 21 mm. long, 300 to 400μ wide at vulva, thinning toward both extremities. Vulva transverse, in posterior part of the body (Fig. 31.14B). Eggs thin-shelled.
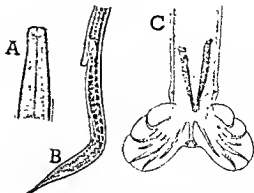
FIG. 34.14 — Amidostomum anseris. (A) Head end. (After Railliet, 1893.) (B) Vulva and tail of female. (After Reinhardt, 1922.) (C) Male tail. (After Railliet, 1893.)

This worm occurs very commonly underneath the horny lining of the gizzard of wild ducks and geese. In the United States it has been reported from domestic geese in the states of New York, Delaware, Pennsylvania, and Washington. No doubt this parasite has a much wider distribution in this country than the present records indicate.

Life history. Eggs pass out in the droppings of infected birds in a partly developed stage, active embryos developing within a few hours and hatching taking place within a few days. Susceptible bird hosts become infected by swallowing with their food or drinking water these infective larvae. Adult worms are recovered within approximately 40 days after the feeding of infective larvae.

Pathology. In the United States, Cram (1926a) reported an outbreak of amidostomiasis in a flock of geese in New York in which a large number of deaths occurred. Heavy losses among geese in Europe have been attributed to this nematode. Young birds show symptoms of loss of appetite, dullness, and emaciation. At necropsy, the lining of the gizzard of a heavily parasitized bird appears necrotic, loosened, often sloughed in places, and is dark brown or black in color in areas adjacent to the site of the worms.

Bunyea and Creech (1926) found a very noticeable leukocytic invasion of the mucosa propria, with eosinophilic cells strikingly predominant.

INTESTINAL TRACT

Ascaridiidae

The members of this family are characterized by having 3 prominent lips, valvulated bulb absent, and preanal sucker sometimes present.

Ascaridia galli (Schrank, 1788)

Synonyms. *Ascaris galli* Schrank, 1788; *Heterakis lineata* Schneider, 1866; *Heterakis inflexa* (Zeder, 1800) Schneider, 1866.

Description. Worms large, thick, yellow-
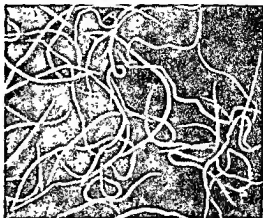
FIG. 34.15 — Roundworms (*Ascaridia galli*) from small intestine of a chicken. (J. E. Ackert.)

ish-white (Fig. 34.15). Head with 3 large lips.

Male 5 to 7.6 cm. long by 490µ to 1.21 mm. wide. Preanal sucker oval or circular, with strong chitinous wall with a papilliform interruption on its posterior rim. Tail with narrow caudal alae or membranes, and 10 pairs of papillae. Spicules equal and narrow.

Female 6 to 11.6 cm. long by 900µ to 1.8 mm. wide. Vulva in anterior part of body. Eggs elliptical, thick-shelled, not embryonated at time of deposition (Fig. 34.16).

This large roundworm is one of the most common nematode parasites of the chicken in the United States and elsewhere. It occurs occasionally in turkeys, but no serious pathologic effects have been reported from its presence in that host.

Specimens of this parasite have been recovered on a number of occasions from broken eggs. The worms had presumably wandered up the oviduct from the intestine via the cloaca with subsequent inclusion in the developing egg.

Life history. The life history is simple and direct. According to Itagaki (1927), the infective eggs which are swallowed by the susceptible host hatch either in the proventriculus or in the duodenum. Ackert (1931) observed that the young larvae, after hatching from the eggs, live free in the lumen of the posterior portion of the duodenum for the first 9 days, fol-

FIG. 34.16 — Ascaridia galli ova.
X400. (E. A. Benbrook, 1928)
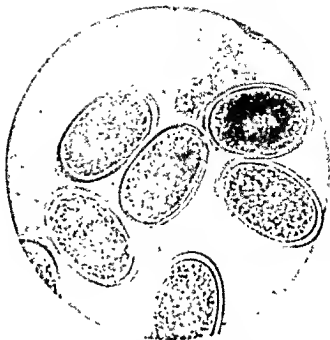
lowing which they penetrate the mucosa and cause hemorrhages. The young worms have again entered the lumen of the duodenum by the seventeenth or eighteenth day and remain there until maturity, which is reached within approximately 50 days after the ingestion of the embryonated eggs. Tugwell and Ackert (1952) reported studies which showed that the Ascaridia larvae may enter the tissues as early as the first day and remain there as late as the twenty-sixth day after infection. The authors stated, however, that the large majority of the larvae spend from the eighth to the seventeenth day in the intestinal mucosa.

Under optimum conditions of temperature and moisture, the eggs in the droppings will develop to infectivity in 10 to 12 days; under less favorable conditions a longer time is necessary. The eggs are quite resistant to low temperatures. Ackert found that, in the early stages of development, eggs survived freezing at −12° to −8° C. for 15 hours, but not for 22 hours; fertile eggs kept at 0° C. for 1 month were unable to reach the infective stage subsequently, whereas eggs from the same culture, kept concurrently at 10° C. for a month, developed normally to the infective stage when incubated at a higher temperature. Fair (1956) recovered Ascaridia galli larvae from experimental birds fed embryonated eggs of this worm which had been exposed continuously to outdoor conditions at Beltsville, Maryland, for 60 weeks. As regards high temperatures, 12 hours' exposure to 43° C. proved lethal for eggs in all stages of development.

Pathology. Ackert (1919) found that chickens infected with a large number of ascarids suffer from loss of blood, reduced blood sugar content, increased urates, shrunken thymus glands, retarded growth, and greatly increased mortality. Droopiness, emaciation, and diarrhea are the common clinical symptoms manifested by heavily parasitized birds (Fig. 34.17A and B).

Experimental evidence is available to show that chickens 3 months or older manifest considerable resistance to infec-
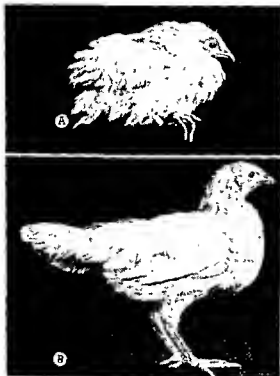
FIG. 34.17 — (A) Chicken infected with large roundworms of intestine. (B) Chicken of same age free of roundworms. (Ackert and Herrick.)

tion with *Ascaridia galli*. Ackert *et al.* (1939) reported that the increased number of goblet cells found in the epithelial lining of the duodenum of chickens at 3 months or older may in some measure be responsible for the greater resistance to this nematode developed by these birds. The age at which the peak of the goblet cell formation occurred was found to correspond very closely to the development of the maximum resistance of the chickens to the growth of the nematodes.

Ackert and Beach (1933) showed that diets consisting chiefly of animal proteins and with little or no plant protein were important in aiding the chicken to build up resistance to infection with ascarids, and that diets consisting chiefly or wholly of vegetable proteins lowered the resistance to ascarid invasion. Alicata (1935), likewise, observed that birds given a diet consisting principally of animal protein con-

centrates developed fewer worms than those which were given a diet low in animal protein. Diets high in vitamins A and B (complex) have been shown to increase the fowl's resistance to *Ascaridia galli*, and diets low in these vitamins definitely favor parasitism.

Experiments conducted by Ackert *et al.* (1935), which extended over a period of years and involved 1,351 chickens, showed that the heavier breeds such as the Rhode Island Reds and White and Barred Plymouth Rocks were more resistant to ascarid infections than the lighter White Leghorns and White Minorcas.

Large roundworms, similar in size and appearance to *Ascaridia galli*, occur in the small intestines of pigeons, guinea fowls, wild turkeys, and other game farm species. *Ascaridia numidae* of the guinea fowl, and *Ascaridia columbae* of the pigeon are shorter and somewhat thicker than *A. galli* of the chicken. The guinea fowl ascarid is the smallest of the three species. *Ascaridia dissimilis* has been found commonly both in the domestic and wild turkey of this country and has been reported by Vigueras (1931) from the domestic turkey in Cuba. This ascarid is very similar in appearance to *A. galli*, but is somewhat smaller. Shillinger (1942) reported *Ascaridia compar* as a parasite of the small intestine of the bobwhite quail in the United States.

The life history of all the above ascarids is probably similar to that of *Ascaridia galli*. Although the life histories of *Ascaridia columbae* and *A. dissimilis* have been shown by the writer to be direct, a detailed account of the hatching of the eggs, the period the larvae spend in the mucosa of the intestine, such as has been recorded for *Ascaridia galli* by Ackert, has not been worked out for these ascarids. The life history of *Ascaridia numidae* has not been experimentally demonstrated.

Birds heavily infected with either *Ascaridia dissimilis*, *A. columbae*, or *A. numidae* are probably affected in a somewhat similar manner as those heavily infected with *A. galli*.
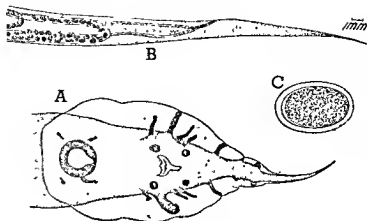
FIG. 34.18 — Heterakis gallinarum. (A) Male tail. (B) Female tail. (After Lane, 1917.) (C) Egg. (After Cram, 1931.)

Heterakidae

Heterakis gallinarum (Schrank, 1788)

Synonyms. *Ascaris gallinae* Gmelin, 1790; *Heterakis papillosa* Railliet, 1885, not *Ascaris papillosa* Bloch, 1782.

Description. Worms small, white. Head end bent dorsally. Mouth surrounded by 3 small, equally sized lips. Two narrow lateral membranes extend almost entire length of body. Esopbagus ending in a well-developed bulb containing a valvular apparatus.

Male 7 mm. to 1.3 cm. long. Tail straight, ending in a subulate point; 2 large lateral bursal wings. Preanal sucker well developed, with strongly chitinized walls and small semicircular incision in posterior margin of wall of sucker. Twelve pairs of caudal papillae; 4 pairs distinctly postanal, 4 pairs of raylike papillae and 2 pairs of sessile papillae adanal, and 2 pairs of raylike papillae in vicinity of sucker (Fig. 34.18A). Spicules dissimilar, the long one 2 to 2.17 mm. long, the short one 700μ to 1.1 mm. long.

Female 1 to 1.5 cm. long. Tail long, narrow, and pointed (Fig. 34.18B). Vulva not prominent, slightly posterior to middle of body. Eggs thick-shelled, ellipsoidal, unsegmented when deposited (Fig. 34.18C).

H. gallinarum has been reported from the ceca of chickens, turkeys, guinea fowls,

bobwhite quail, pheasants, and many other birds.

Life history. The eggs pass out in the feces in an unsegmented state. In approximately 2 weeks or less, under favorable conditions of temperature and moisture, these eggs will have reached the infective stage. When the latter are swallowed by a susceptible host, the embryos hatch from the eggs and develop to adult worms in the ceca. Roberts (1937) stated that the eggs hatched in the upper part of the intestine, and at the end of 24 hours the majority of the young worms have reached the ceca. Aside from a short period in the cecal mucosa, 2 to 5 days, according to Uribe (1922), the entire life of the cecal worm is spent in the lumen of the cecum. At necropsy, the majority of the adult worms are found in the tips or blind ends of the ceca. Earthworms may ingest the eggs of the cecal worm and may be the means of causing an infection in poultry, as the latter are very fond of earthworms.

Pathology. Riley and James (1922) observed that the ceca of experimentally infected birds showed marked inflammation and thickening of the walls.

The chief economic importance of the cecal worm lies in its role as a carrier of the blackhead organism, *Histomonas meleagridis*. Graybill and Smith (1920) demonstrated by experimental methods that blackhead may be produced in sus-

ceptible birds by feeding embryonated eggs of *Heterakis gallinarum* taken from blackhead-infected birds. These authors were of the opinion that the cecal worms lowered the resistance of the host to such a degree that the protozoan parasites already present were able to multiply to disease-producing proportions. Tyzzer (1926) presented evidence which indicated that the protozoan parasite is incorporated in the worm egg; however, he was unable to demonstrate the presence of the protozoan parasite within the egg. Kendall (1959) saw organisms which resembled *Histomonas* in a young larval *Heterakis gallinarum* worm. Gibbs (1962) identified the *Histomonas* organism in the gut wall and in the reproductive system of the male and the female and in the developing eggs of this cecal worm. Farr (1956) recovered *Histomonas* organisms from experimental birds fed droppings containing *Heterakis gallinarum* and other nematode eggs that had been exposed continuously on soil to natural weather conditions from 17 to 66 weeks, inclusive. Several of the test birds which had been fed this material died of blackhead disease.

Two other species of cecal worms, *Heterakis beramporia* and *Heterakis isolonche*, occur in the ceca of chickens and pheasants, respectively. So far as is known, the former species does not occur in birds of the United States. However, the latter species has been found in pheasants in Pennsylvania and Connecticut. Both of these heterakids produce nodules in their respective hosts.

Subulura brumpti (Lopez Neyra, 1922)

Subulura brumpti has been reported by Alicata (1940) to be a common pinworm of chickens in the Hawaiian Islands. Cram (1926b) and Dikmans (1929) reported this pinworm as occurring in the turkey in Puerto Rico. Foster (1939) collected it from the fowl in Panama, and Ward (1915) listed it as a parasite of the quail in Mississippi (Fig. 34.19A and B).

No evidence that the larvae penetrated the cecal wall of the bird host for any
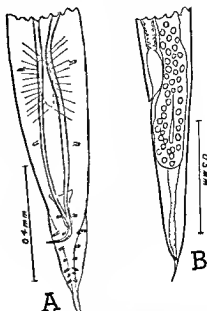
FIG. 34.19 – *Subulura brumpti*. (A) Posterior end of male, ventral view. (B) Posterior end of female, lateral view. (From Cuckler and Alicata, 1944.)

part of its development, nor that they produced any extensive inflammatory tissue reaction was reported by Alicata (1940). Various insects, such as beetles and earwigs, serve experimentally as intermediate hosts of this cecal worm.

Another cecal worm, *Subulura strongylina*, has been reported by Venard (1933) from the bobwhite quail in Ohio; by Cram (1927) from the chicken and guinea fowl in Puerto Rico; by Dikmans (1929) from the guinea fowl and by Van Volkenberg (1938) from poultry in Puerto Rico.

Trichuridae

For family diagnosis, see page 974.

Capillaria obsignata Madsen, 1915

Synonyms. *Capillaria dujardini* Travassos, 1915, nec Travassos, 1914; *Capillaria columbae* (Rudolphi, 1819) of Graybill, 1924.

Description. Worms hairlike.

Male 8.4 mm. to 1.2 cm. long by 49 to 55μ wide. Cloacal aperture almost terminal, with a small bursal lobe on either
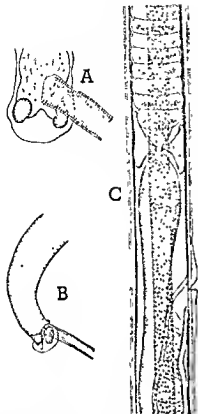
FIG. 34.20 — Capillaria obsignata. (A) Ventral view, and (B) Lateral view, of male tail. (After Graybill, 1924.) (C) Region of vulva. (After Eberth, 1863, slightly modified.)

side, the two lobes connected dorsally by a delicate bursal membrane (Fig. 34.20A). Spicule sheath with transverse folds; spicule 1.1 to 1.58 mm. long.

Female 1 to 1.8 cm. long by approximately 80μ wide. Vulva on slight prominence, slightly posterior to union of esophagus and intestine (Fig. 34.20C). Eggs slightly brownish, lemon-shaped, thick-shelled.

This hairworm occurs in the small intestine of the domestic and wild pigeon, chicken, and turkey in the United States.

Life history. Capillaria obsignata has a direct development. The freshly deposited eggs are unsegmented and require from 6 to 8 days to develop completely formed embryos. The embryos do not escape from the eggs until after they have been swal-

lowed by a susceptible host. The larvae enter the mucosa of the duodenum and apparently complete their development there. A few sexually mature adults were removed by the writer from the small intestine of a pigeon necropsied 19 days after the ingestion of the embryonated eggs, and a large number of similarly developed adults were removed from the small intestine of a pigeon necropsied 26 days after infection. Fecal examination of the latter pigeon at the time of necropsy showed the presence of eggs.

It has been experimentally demonstrated that pigeons, when once infected with Capillaria obsignata and held under conditions designed to preclude reinfection, will remain infected for about 9 months.

Capillaria obsignata has been reported from the small intestines of chickens and turkeys raised under natural conditions. Graybill (1921) stated that as a result of many necropsies of chickens and turkeys, this roundworm was never observed in these birds in large numbers. Wehr (1939) was successful in obtaining natural infections in chickens and turkeys, but no heavy infections were encountered in the latter.

Pathology. Birds heavily infected with Capillaria obsignata spend much of their time apart from the rest of the flock, huddled on the ground, underneath the roosts, or in some corner of the room. Such birds show definite symptoms of emaciation and diarrhea. The feathers around the vent frequently appear ruffled and soiled, and the skin and visible mucous membranes are more or less pale. Death is often the result of heavy infections. Levine (1938) reported that the first clinical symptoms of infection in chickens appeared on the twelfth day after experimental inoculation of embryonated eggs. At this time the feces contained much pinkish material composed of mucus, necrosed epithelial cells, and numerous erythrocytes, granulocytes, and lymphocytes. From the twelfth to the sixteenth days the feces of the birds were watery and contained large quantities of epithelium and inflammatory exu-

date which was being eliminated from the intestinal tract. Following this period, most of the infected birds regained their normal appearance, and the feces became normal. However, many of the birds lost weight steadily, became extremely emaciated, and either died or were destroyed because of a weakened condition. In fatal and in advanced cases of infection, the intestines showed extensive destruction of the mucosa, often with complete sloughing of the mucous membrane. The intestines usually contain a large quantity of fluid. In nonfatal experimental cases the intestinal wall was thickened considerably owing to the edematous infiltration.

Another threadworm, *Capillaria caudinflata*, has been occasionally found in chickens, turkeys, and pheasants in the United States. This species of capillarid worm may be differentiated easily from other species of the same genus found in poultry of this country by the presence on the male tail of two large lateral transparent membranes just anterior to the cloacal aperture, and on the female of a membranous tubular or trumpet-shaped projection in the region of the vulva.

Allen and Wehr (1942) experimentally infected turkeys with *Capillaria caudinflata* by feeding to them earthworms of the species *Allolobophora caliginosa* which were removed from poultry yards in which were confined turkeys known to harbor this threadworm. Morehouse (1944) demonstrated that the above earthworm was an essential intermediate host for the successful transmission of *Capillaria caudinflata* from turkey to turkey. Attempts by Wehr to transmit this threadworm by using species of the earthworm, *Eisenia foetida* and *Lumbricus terrestris*, were unsuccessful. Wehr and Allen (1945) introduced directly into the alimentary tracts of several earthworms, *Eisenia foetida*, embryonated eggs of *Capillaria caudinflata*, and later adults of this worm were recovered at necropsy from turkeys to which these earthworms had been fed.

Barile (1912) found these worms in turkeys showing hemorrhagic, croupous enteritis but was unable to state positively whether the worms were responsible for this pathological condition. Baker (1930), in connection with frequent findings of this worm in the province of Quebec, Canada, noted that the worms were associated with ulcerous patches varying in size from pin-point areas to greatly extended and hardened areas. An infected chicken observed by Graham *et al.* (1929) in Illinois showed weakness, anemia, and emaciation before death. At necropsy, the intestine just anterior to the ceca was markedly dilated, with a follicular diphtheritic enteritis present.

Trichostrongylidae

For family diagnosis, see page 964.

Ornithostrongylus quadriradiatus (Stevenson, 1904)

Synonym. *Strongylus quadriradiatus* Stevenson, 1904.

Description. Worms delicate, slender, red when freshly collected, apparently from ingested blood in intestine. Cuticle about head inflated to form vesicular enlargement (Fig. 34.21A).

Male 9 mm. to 1.2 cm. long. Bursa bilobed, with no distinct dorsal lobe. Dorsal ray much shorter than other rays, not extending halfway to bursal margin, bifurcating near its tip to form 2 short tips, and a stumpy process present on each side near base of ray. Spicules equal, 150 to 160μ long, somewhat curved, each terminating in 3 pointed processes (Fig. 34.21 B). Telamon 57 to 70μ long, with 2 longitudinal processes extending backward and forward along dorsal wall of cloaca, and 2 lateral processes forming a partial ring through which the spicules protrude.

Female 1.8 to 2.4 cm. long. Vulva near end of tail. Vagina short, followed by 2 powerful muscular ovejectors. Tail tapers to a narrow, blunt end, bearing a short spine. Eggs segmenting when deposited.

This bloodsucking nematode occurs in the small intestine of pigeons and mourning doves in the United States.

Life history. The oval, thin-shelled eggs
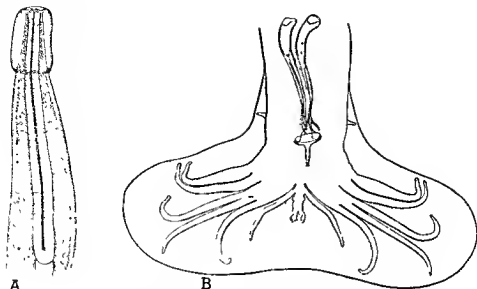
FIG 34.21 — Ornithostrongylus quadriradiatus. (A) Anterior end. (B) Caudal bursa of male. (After Stevenson, 1904.)

are voided in the droppings and hatch in approximately 19 to 24 hours under favorable conditions of moisture and temperature. After escaping from the egg, the young larva molts twice within the next 3 or 4 days. It has now reached the infective stage. When the infective larva is swallowed by a pigeon or other susceptible host, it grows to maturity in the small intestine. The female worm begins to deposit eggs in 5 or 6 days following ingestion of the larva.

Pathology. Stevenson (1904) observed that this parasite was the cause of many deaths among a flock of fancy pigeons in Washington, D.C. Le Roux (1926, 1930) mentioned this roundworm as having caused serious losses in a flock of valuable imported pigeons. Vigueras (1929) reported similar losses among pigeons in Cuba, and Kamarov and Beaudette (1931) attributed large numbers of deaths among squabs as having been due to this bloodsucking parasite. These investigators are agreed that deaths among the birds were attributable principally to a catarrhal enteritis and a loss of blood due to hemorrhage.

Birds heavily infected with Ornithostrongylus quadriradiatus behave much the same as birds heavily parasitized with other bloodsucking parasites. They become droopy, remain squatted on the ground or floor, and if disturbed, they try to move but usually tip forward on the breast and head. Food is eaten sparingly and is frequently regurgitated, along with bile-stained fluid. There is a pronounced greenish diarrhea, and the bird gradually wastes away. Symptoms of difficult and rapid breathing usually precede death. The intestines of fatally infected birds are markedly hemorrhagic and have a greenish mucoid content, with masses of sloughed epithelium (Fig. 34.22).

Trichostrongylus tenuis (Mehlis, 1846)

Synonyms. Strongylus tenuis Mehlis, 1846 (in Creplin, 1846); Strongylus pergracilis Cobbold, 1873; Trichostrongylus pergracilis (Cobbold, 1873) Railliet and Henry, 1909.

Description. Worms small and slender. Body gradually attenuated in front of genital opening. Mouth surrounded by 3 small, inconspicuous lips. Cuticle of an-
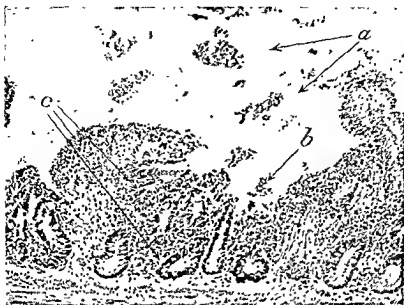
FIG. 34.22 — Section of duodenum of pigeon during later stage of infection with *Ornithostrongylus quadriradiatus*, showing: (a) Sloughing of mucosa; (b) Necrotic areas; and (c) Lymphocytic infiltration. ×150. (After Cuvillier, 1937.)

terior end of body lacking conspicuous striations for a distance of about 200 to 250μ from extremity, then with distinct serrated appearance for a distance of about 1 to 2 mm. more.

Male 5.5 to 9 mm. long by 48μ wide near center of body. Cuticle inflated on ventral surface just anterior to bursa. Bursa with one dorsal and two lateral lobes, the dorsal one not distinctly marked off from the lateral. Each lateral lobe supported by 6 rays (Fig. 34.23A). The dorsal ray bifid at its distal third, and each of these divisions again bifid and very finely pointed (Fig. 34.23B). Spicules dark brown in color, slightly unequal in length, the longest 120 to 164μ long, the shortest 104 to 150μ long: both much twisted, especially at distal ends, and provided with an earlike structure on proximal end (Fig. 34.23C). Both spicules apparently surrounded in distal two-thirds by a thin membrane extending for a short distance beyond distal ends. Gubernaculum strongly cuticularized along margins, spindle-shaped in ventral and dorsal views (Fig. 34.23D and E).

Female 6.5 mm. to 1.1 cm. long by 77 to 100μ wide at level of vulva. Vulva in posterior part of body, with crenulated edges. Uteri divergent. Eggs thin shelled.

It was concluded by Cram and Wehr (1934), as the result of a critical study of a large number of specimens of the genus *Trichostrongylus* collected from the ceca of both American and European domestic and wild game birds, that the material thus examined represented only one species instead of two, as previously suspected. Although *Trichostrongylus pergracilis* had been described from the ceca of many birds, the differences between the description of this species and that of *T. tenuis* were inconsequential and not of specific value. As a result of the above study, therefore, the authors concluded that *T. pergracilis* and *T. tenuis* were identical morphologically, and since the specific name *tenuis* had priority over *pergracilis*, the former name was accepted as the valid name.

In the United States, *T. tenuis* has been collected from the pheasant, *Phasianus colchicus;* the blue goose, *Chen caerules-*
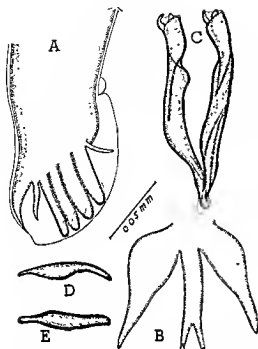
FIG. 34.23 – Trichostrongylus tenuis. (After Cram and Wehr, 1934.) (A) Bursa, lateral view, semidiagrammatic. (B) Dorsal and externodorsal rays of bursa, showing variation which may occur in length of latter. (C) Right and left spicule, ventral view. (D) Gubernaculum, lateral view. (E) Gubernaculum, dorsal view. A and B from European partridge, C to E from red grouse. Scale refers to camera lucida drawings, B to E, inclusive.

cens; the Canadian goose, Branta canadensis; the domestic goose, Anser anser domesticus; the guinea fowl, Numida meleagris; the chicken, Gallus domesticus; the turkey, Meleagris gallopavo; and the bobwhite quail, Colinus virginianus. The red grouse, Lagopus scoticus, and the European partridge, Perdix perdix, are the only two hosts from which T. tenuis has been collected in Europe. According to Cram (1931a), this trichostrongyle occurs widely among the quail of the southeastern United States.

Life history. This worm has a direct life history. The eggs hatch within 36 to 48 hours after they have been passed in the droppings of the infected bird. The larvae become infective within approximately 2

weeks following expulsion of the eggs in the droppings. Within this time the larvae have molted twice. When the latter are picked up by a susceptible host, the infective larva molts twice more within the ceca of the bird before finally becoming an adult.

Trichostrongylus tenuis from pheasants has been successfully transmitted to the domestic turkey, guinea fowl, and chicken.

Pathology. T. tenuis produces definite clinical symptoms when present in large numbers. The changes in the ceca consist of a thickening and a reddening of the walls, and small hemorrhages are sometimes present. Loss of weight, anemia, and chronic toxemia have been reported as symptoms resulting from heavy infections.

Strongyloididae

Members of this family are characterized by having an alternation of generations, the free-living generation consisting of males and females while the parasitic generation consists of hermaphroditic females only.

Strongyloides avium Cram, 1929

Description. Parasitic generation, consisting of parthenogenetic females only, in intestine of avian host, and free-living generation, consisting of both males and females, in soil.

Parasitic adult. 2.2 mm. long by 40 to 45μ wide. Vulva with projecting lips, located 1.4 mm. from head end. Uteri divergent from vulva; ovaries recurrent in simple "hair-pin bends," their course not sinuous. Eggs with very thin shells, segmenting when deposited.

Cram (1929) reported the occurrence of this extremely small roundworm from the ceca of chickens in Louisiana. Later (1936h), this same author reported this species from the ceca and small intestines of chickens in Puerto Rico. The junco, Junco hyemalis hyemalis, in Virginia, and the coot, Fulica americana, in North Carolina have been found to harbor natural infections of this parasite.

Life history. The eggs hatch soon after

being passed in the droppings, sometimes as soon as 18 hours. The young worms develop in the soil to adult males and females. *Shortly thereafter the females give rise to young, which feed, molt, and develop into other adult free-living males and females, or they may transform into another type of larvae known as the infective larvae.* When these infective larvae are swallowed by a susceptible host, infection results. Unlike most species of nematodes, the parasitic cycle of *Strongyloides avium* consists of females only, no parasitic males having been found.

Pathology. During the early or acute stage of the infection the walls of the ceca are greatly thickened; typical pasty *cecal contents almost disappear, the dis-*charge being thin and bloody. If the fowl survives this acute stage, the ceca gradually become functional again, and the thickening of the walls decreases. Young birds suffer most from infections with this worm. If the infection is light or if the birds are adults, little, if any, clinical effect has been noted.

CONTROL OF POULTRY NEMATODES

Prevention. Preventive measures for the control of poultry roundworms have been developed along the lines of sanitation, *hygiene, and management, and it is along* these lines that the greatest progress has been made.

The proper selection of a permanent site for the poultry runs is one of the first essentials to the maintenance of health among the birds. The land should be sloping and of a sandy or gravelly nature to provide for proper drainage. If the soil is heavy, or the lay of the land is such as to render natural drainage impossible, artificial drainage should be provided. The nonparasitic stages of helminth parasites require moisture for their proper development. Therefore, the presence of surface water, which birds are apt to drink, must be regarded as unhealthful, and provisions should be made to eliminate it as soon as possible. Damp places and water holes are ideal breeding places for many of the intermediate hosts of poultry nematodes.

The practice of rotating the birds from one area of land to another in order to reduce parasitism among the birds has been followed with reasonably good success in some sections of the country. The four-yard system is the one most widely advocated and probably the one best suited for general use. A given area of land—the amount depending upon the number of birds raised—is cross-fenced so that it is divided into four equal lots. The shelter or house is placed in the center of the plot and so constructed that a door opens into each pen. The birds are rotated from one pen to another, keeping them in each pen no longer than 2 or 3 *months. After removing the birds from* one of the pens, the ground in that pen may be prepared for planting to some green crop or left undisturbed to undergo self-sterilization by exposing the infected droppings to the direct action of the sun, wind, and cold. The planting of the yards to a permanent or temporary crop serves a twofold purpose: (1) It furnishes abundant green food for the growing birds, and (2) there is some evidence to indicate that birds are less likely to pick up contaminated soil when plenty of green food is available, thus reducing parasitism in the birds. The house and adjacent grounds should be cleaned as often as is deemed necessary to maintain good sanitation. The practice of removing the soil about the house to a depth of 6 to 8 inches and replacing it with sand or coarse ashes has sometimes been followed. During the summer months, birds spend a great deal of their time in the shade of the house, and it is necessary that extra precautions be taken to improve the sanitary conditions around the house.

The frequent removal of the droppings and the proper disposal of them cannot be recommended too highly. (See discussion of manure disposal under control of cestodes, p. 1030.)

The raising of the different species of birds together or in close proximity to each other is a dangerous procedure as re-

gards parasitism. Ransom (1921) showed by careful investigations that adult turkeys served as carriers of gapeworms in transmitting gapeworm disease to little chicks and that the older chickens were almost entirely insusceptible to infection with the above worms. Adult turkeys carrying natural infections of gapeworms apparently suffer only slightly, while young chicks and turkey poults suffer very severely from infections with gapeworms.

It is also dangerous for turkeys to associate with chickens. Tyzzer (1928) established the fact that blackhead occurs in young and old chickens, the latter usually recovering from the disease without suffering a great deal. However, this same investigator also demonstrated that the recovered chickens remained carriers of the blackhead organism for an indefinite period, and that turkeys contract blackhead by exposure to infected poultry or runs occupied by the latter.

It follows from the work of Ransom, Tyzzer, and others that anyone wishing to raise poultry would do well to decide in the beginning to raise turkeys or to raise chickens but not to raise both on the same land or in close proximity to each other. Should a person desire to raise both chickens and turkeys, he should keep the two kinds of birds on ranges well separated from one another

It is dangerous too for turkeys and chickens to associate with guinea fowls. Wehr (1959b) showed experimentally that the guinea fowl is susceptible to infection with the poultry gapeworm, *Syngamus trachea,* at any age and that this bird may carry the parasites for as long as 98 days.

Treatment Satisfactory remedial agents have been discovered for the removal of the poultry gapeworm, *Syngamus trachea,* the large intestinal roundworm of the chicken, *Ascaridia galli,* and the cecal worm, *Heterakis gallinarum,* but equally effective drugs for the removal of the other poultry nematodes are lacking.

Wehr *et al.* (1939) found that the compound, barium antimonyl tartrate, successfully removed a very high percentage of gapeworms from chicks, turkey poults, and adult turkeys when administered as an inhalant.

For treatment the birds are placed in a suitable container, such as a tight box. The drug is introduced into the box as a very fine powder by means of a dust gun. Because of its fluffiness, the powder remains suspended in the air for a long time. As the dust-laden air is breathed in by the infected birds, the fine particles of barium antimonyl tartrate apparently adhere to the moist surfaces of the worms and act as a contact poison.

The size of the dose of the powder to be administered is determined by the cubic capacity of the treatment box. One ounce of barium antimonyl tartrate has been found to be sufficient for a box having a capacity of 8 cubic feet. Approximately one-third of the powder is introduced into the treatment box at the first operation. If the box is of a convenient size to lift, it is tilted slowly from side to side several times. Tilting the box causes the birds to flap their wings, thus aiding in redispersing any powder that may have settled on the feathers of the birds or on the floor of the box. Tilting also causes the birds to breathe deeper and more frequently. Deep breathing is necessary to bring the powder in contact with the worms that may be found in the lower part of the trachea. In the case of mature birds, when the treatment box is likely to be too large and heavy to tilt by hand, a small electric fan may be placed on the floor of the box to keep the powder agitated during the process of treatment. After about 5 minutes, one-half of the remaining powder is blown into the box and the tilting or the use of the fan is repeated. The remaining one-third of the powder is introduced into the treatment box about 15 minutes following the introduction of the first one-third, and the box is again tilted or the fan used. The birds are released 5 to 10 minutes after the last of the powder has been blown into the box.

Rectal injections of a mixture of oil of chenopodium and of olive oil or cottonseed oil, given with a hard rubber syringe, in doses of 0.1 cc. in 5 cc. of the oil in case of birds weighing 1.5 pounds and double this amount of chenopodium and oil for adult birds weighing 3 pounds or over, have been found by Hall and Shillinger (1923b) to be approximately 90 per cent efficacious for the removal of the cecal worm of chickens and turkeys. McCulloch and Nicholson (1940) reported that phenothiazine, when given either in repeated or single doses, was very effective for the removal of the cecal worm from chickens. Doses ranging between .05 and 0.5 grams were found to be the most satisfactory individual doses. These authors found repeated doses and the administration of the drug in individual capsules to be slightly more satisfactory, although they indicated that flock medication appeared to be practical. They experimentally determined that doses up to 500 times the smallest amount found to be therapeutically effective had no apparent harmful effect on the birds; such massive doses also had no antiheterakid effect. The administration of phenothiazine in doses of 0.5 grams per bird had no appreciable effect on a flock in egg production and was not followed by enteritis or other digestive disturbances, except for a slight softening of the feces 24 hours after treatment. For flock treatment one pound of powdered phenothiazine is mixed with as much wet mash as 900 to 1,000 birds will consume in approximately one hour. The mash and the phenothiazine should be thoroughly mixed when dry, then moistened to increase palatability. Great care must be taken that there are plenty of feed hoppers to permit all birds in the flock to eat at the same time.

Birds given phenothiazine should be confined to the house. Feed should be withheld for a few hours or half a day until the birds become quite hungry. Worms will be eliminated in the droppings during the 2 or 3 days following the treatment. The house should then be thoroughly dry cleaned and clean litter added. Birds that are given access to yards or the range during treatment should be removed to new soil not later than one week after treatment, and the soil exposed to action of sun and wind for several months if possible.

Hall and Shillinger (1923a) reported that carbon tetrachloride failed to remove any large roundworms from one chicken treated with 1 cc. per kilo of body weight, but removed all the worms present in three cases when administered at the rate of 2, 4, and 5 cc. per kilo of body weight. Ackert and Graham (1935) found that carbon tetrachloride was highly efficacious in removing the large intestinal roundworm from young chickens at a dose rate of 4 cc. per kilo with apparently no ill effects.

It has been known for many years that nicotine possesses a high nematocidal action against the large roundworm of poultry. However, because of its toxicity to the fowl, its use for the control of this parasite was temporarily delayed.

Herms and Beach (1916) were the first to employ tobacco stems for the removal of poultry roundworms. They found that by soaking chopped tobacco stems for 2 hours and mixing the stems and the liquid with about one-third of their normal ration of mash, many roundworms were removed. The birds were fasted for about 24 hours before they were given the medicated mash.

Freeborn (1923), of the University of California Agricultural Experiment Station, conceived the idea of mixing nicotine sulphate (Black Leaf "40") with an earthen material known as Lloyd's alkaloidal reagent, thereby rendering much less toxic a substance which by itself would not be safe to use. The mixture, which contained 6.6 cubic centimeters of nicotine sulphate and 16 grams of Lloyd's alkaloidal reagent, was placed in capsules, each capsule containing approximately 350 to 400 milligrams, and administered individually to the birds. This formula became known as the University capsule.

The introduction of this capsule marked a renewed interest in the treatment of fowls for the removal of the large intestinal roundworm. However, it was soon discovered that the gelatine capsule containing the mixture was soluble in the secretions of the upper part of the digestive tract, and toxic symptoms and, in some cases, death resulted. Moreover, necropsies on some of the treated birds revealed the presence of numerous roundworms, indicating that the dose of nicotine in the University capsule was not sufficient to remove all the worms satisfactorily

Davis (1940) stated that it was possible to mix nicotine with an organic colloidal material (name not given) to obtain a mixture which was nonlethal to the animal to which it is administered even though the amount of nicotine is in excess of a lethal dose. The use of such a mixture, he stated, satisfactorily removed roundworms from chickens, provided 70 to 80 milligrams of nicotine were administered at a single dose He further stated that it was possible in the mixing of the nicotine and the organic colloidal material to so regulate the release of the alkaloid (nicotine) that the greatest amount could be liberated where it was most needed. In the case of the intestinal roundworm, which is found to be most numerous in the anterior portion of the small intestine, the liberation of the drug would have to be delayed until after it passed through the gizzard.

Levine (1936) administered a mixture containing one-fourth pound Black Leaf worm powder (a 5 per cent nicotine compound composed of nicotine sulphate mixed with a special fuller's earth) and 5 pounds of mash to 45 pullets Feed was withheld from the birds overnight. The birds promptly cleaned up the mash the next morning. Two hundred and eighty-four worms were removed by the treatment; none was found at autopsy.

Guthrie and Harwood (1942) conceived the idea of mixing phenothiazine and nicotine-bentonite (a claylike material) and administering the mixture for the removal of both *Heterakis gallinae* and *Ascaridia galli* from chickens. Tablets containing 33 parts phenothiazine, 66 parts nicotine-bentonite (5 per cent nicotine), and 1 part sodium stearate removed 83.7 per cent of 1,012 *Heterakis* and 96.2 per cent of 131 *Ascaridia*. When administered separately and in equivalent amounts to infected chickens, the phenothiazine removed 89.9 per cent of 675 *Heterakis* and 48.2 per cent of 110 *Ascaridia*; the nicotine-bentonite removed 10.1 per cent of 1,246 *Heterakis* and 85.2 per cent of 149 *Ascaridia*.

Harwood and Stunz (1945) found that phenothiazine and nicotine-bentonite mixture gave good results in removing *Heterakis gallinae* and only fair results in removing *Ascaridia dissimilis* from the turkey.

Scientists of the Bureau of Animal Industry demonstrated that the feeding of a medicated mash containing 15 grams of nicotine sulphate (Black Leaf "40"), 151 grams of phenothiazine, 287 grams of bentonite, and 44 pounds of chick feed for 3 consecutive days at intervals of 3 weeks to chickens held continuously on worm-infected soil maintained a low level of parasitism in the treated birds.

Riedel (1951) investigated the anthelmintic value of Caricide (1-diethylcarbamyl-4-methylpiperazine hydrochloride) as a flock treatment in chickens infected with *Ascaridia galli*; he found that feeding a mash containing 1.0 per cent Caricide for a 2-week period and administration of an Epsom salt purge at the end of the first and second weeks eliminated 89.2 per cent of the worms, while none of the untreated birds passed worms. A third group of birds, treated similarly with Caricide but not purged, eliminated 72.0 per cent of their worms.

Piperazine and its derivatives have been recently shown to be highly effective as anthelmintics in man and other animals Bradley (1955) reported a very high recovery of *Ascaridia galli* in two tests in which 15,600 8-week-old broiler chicks were given a water solution containing 8

gm. of piperazine citrate per 1 gallon of drinking water for 60 hours, and 17,900 6-week-old birds were given a similar solution containing 6 gm. of piperazine citrate for 24 hours. Shumard and Eveleth (1955) reported that piperazine citrate, when administered at the rate of 8, 10, and 16 gm. per 1 gallon of drinking water for 1 to 4 days, effectively removed all *Ascaridia* but not *Heterakis*. Vianello and Vicenzoni (1955) administered piperazine citrate to fowls in doses of 300 to 400 mg. per kg. body weight and reported the complete disappearance of both mature and immature *Ascaridia*. No previous fasting was necessary, and the drug was well tolerated. Horton-Smith and Long (1956) tested three piperazine compounds (piperazine carbon bisulphide, piperazine adipate, and piperazine citrate) against *Ascaridia galli* in chickens and reported that all adult worms were completely eliminated. The compounds were administered as single doses varying from 100 to 500 mg. per kg. body weight. Two of the drugs (piperazine adipate and piperazine citrate) removed from 80 to 100 per cent of the *Ascaridia* when incorporated in the feed at the rate of 300 mg. in 100 gm. wet mash or 300 mg. per 200 ml. of drinking water. Colglazier, Foster, Enzie, and Thompson (1960) reported that 1 gm. doses of phenothiazine removed 94 per cent of the *Heterakis* and 24 per cent of the *Ascaridia* present. Piperazine citrate, given by capsule in single doses containing 200 mg. of piperazine, removed 66 per cent of the *Heterakis* present. Doses of 100 mg. and 200 mg. piperazine were both effective against *Ascaridia*. Single 1 gm. doses of 7:1 mixture of phenothiazine and piperazine removed 94 per cent of the *Heterakis* and 100 per cent of the *Ascaridia*. Comparable results were obtained against both species with a 0.75 gm. dose of a 12:1 mixture of the two chemicals. Whitney (1957) reported that piperazine citrate effectively removed *Ascaridia columbae* from pigeons by withholding drinking water overnight and giving a solution containing 8 gm. of the compound to each

gallon of water the next morning. The solution was replenished each morning for the next 3 days and clean water added at noon on the third day. No ill effects, except a slight nausea, were noted.

None of the piperazine compounds is particularly dangerous to the administrator or its recipients. Skin contact over a long period of time may produce a mild irritation, but washing of the exposed areas with copious amounts of water will alleviate the condition.

Most of the piperazine derivatives have a broad safety margin and, therefore, a very low toxicity to the host. On *Ascaridia*, these compounds exert a narcotizing effect, thus enabling the worms to be removed by means of natural peristalsis. The worms are expelled alive and may be seen wriggling if observed soon after expulsion.

The sterilization by means of fumigants of soil contaminated with the eggs and larvae of poultry parasites is receiving some attention. It has been found that methyl bromide is highly effective against ova of some of the common nematode parasites of swine (Andrews *et al.*, 1945) and of poultry (Clapham, 1950). Earthworms and other arthropods inhabiting the soil are also readily killed by methyl bromide fumigation.

For the treatment of the eyeworm, Sanders (1929) recommended a modification of that advocated by Wilcox and McClelland in 1913. The eye is first anesthetized by means of a local anesthetic. The worms are exposed by lifting up the nictitating membrane and one or two drops of a 5 per cent solution of creolin is placed directly on the worms. The eye is then immediately irrigated with pure water to remove the excess creolin solution. Inasmuch as the worms are killed immediately upon contact with the creolin solution, the irrigating of the eye does not interfere with the effectiveness of the treatment. Within 48 to 60 hours after the treatment, the eyes will begin to show improvement, provided the damage has not been too great.

Emmel (1939) reported that the feed-

ing of regular mash to which 5 per cent of flowers of sulphur had been added seemed to benefit turkeys infected with *Capillaria contorta*. At the end of 3 weeks' treatment, he stated that "recovery occurred in all affected birds which were able to eat when treatment was started."

Thienpont and Mortelmans (1962) reported that capillariasis in pigeons and chickens (*Capillaria obsignata*) could be controlled by the administration of 1 cc. of a 10 per cent methyridine solution subcutaneously in the pectoral region or into the leg (pigeon) and dorsal region between the wings (chickens). The writers stated that this drug must be administered with great care as (1) spilling of the drug on the skin may produce a small lesion, (2) nausea, slight ataxia, and incoordination were observed to some degree even with subeffective doses, and (3) death may sometimes result. The drug had no marked effect in coccidiosis and trichomoniasis, and was only slightly effective against *Ascaridia*.

Clarke (1962), following a number of investigations in the use of haloxon against *Capillaria* infection in chickens, reported that individual doses of 25 and 50 mg. per kg. body weight practically eliminated all the worms. However, it was necessary to remove the birds from the infected environment immediately after dosing to prevent reinfection. It was suggested that the most suitable time to treat was immediately prior to movement from the rearing to the laying quarters.

Poultry manure serves as a breeding place or feeding grounds for a large number of arthropods or their progeny, many of which serve as vectors of those species of poultry parasites that require an intermediate host in order to complete their development. It is here that the eggs of these parasites develop to the stage infective to the intermediate hosts.

Circumstances at times arise which do not permit the disposal of poultry manure in a sanitary manner. In order to surmount such an obstacle, the possibility of treating the accumulated manure with insecticides for the destruction of arthropods has received some attention.

Work in Hawaii on the control of arthropods breeding in poultry manure by Tanada *et al.* (1950) and Kartman *et al.* (1950) indicates that kerosene solutions of DDT and chlordane at 1 per cent and acetone solutions of benzene hexachloride at below 1 per cent gamma concentration were highly effective under simulated natural conditions. (See Control of Tapeworms, p. 1029).

Acanthocephalids

The Acanthocephala or thorny-headed worms are parasites occurring as adults in the intestinal tract of vertebrates. In form they are elongate, roughly cylindrical, or spindle-shaped. Several distinct body regions are recognizable: retractile proboscis armed with hooks, a neck, and a body proper. The retractile proboscis bears always a considerable number of recurved hooks which are arranged in rows. The number, form, and arrangement of the hooks are valuable diagnostic characters. The body proper forms the major portion of the worm. It is usually unarmed but may bear small spines of definite form and arrangement on some portion of the external surface. This group of worms is deprived of a digestive tract. Nutrition is thus provided for entirely by absorption through the body wall. The sexes are separate in all cases. The male is smaller and more slender than the female and often distinguished externally by a bell-shaped bursa that surrounds the genital pore.

So far as known, all species of *Acanthocephala* require one or more intermediate hosts before reaching a stage of development where they are infective for the final host. Various arthropods, snakes, lizards, and amphibians serve as hosts of the larval stages of these parasites.

Only three species of thorny-headed worms have been reported as parasites of domestic poultry in North America, two of these as immature forms.

Oncicola canis (Kaupp, 1909)

Oncicola canis (Kaupp, 1909) was found in about 10 per cent of the young turkeys around San Angelo, Texas, by Price (1929). The worms were encysted under the epithelial lining of the esophagus in numbers varying from a few to 100 or more. They were reported as the possible cause of death (Fig. 34.24A, B, C, and D).

The adults of this parasite occur in the dog and coyote. The presence of larval forms of this parasite in young turkeys suggests that such occurrences are accidental, the young worms encysting when taken into an unsuitable host.

Plagiorhynchus formosus Van Cleave, 1918

An immature male and two female specimens of *Plagiorhynchus formosus* Van Cleave, 1918, were reported by Jones
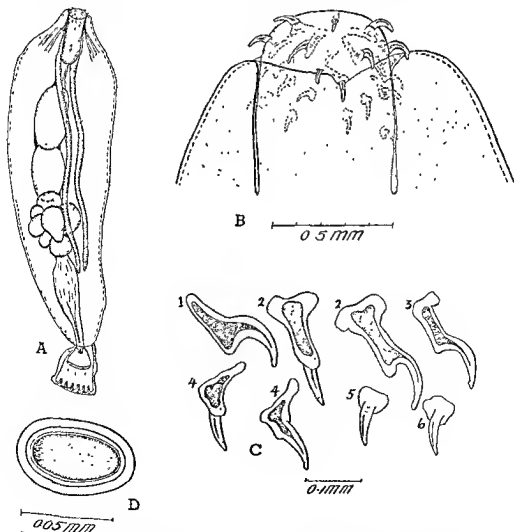
FIG. 34.24—Oncicola canis. (A) Male showing reproductive organs. (B) Proboscis. (C) Hooks from proboscis (numerals indicate row). (D) Egg. (From Price, 1926.)
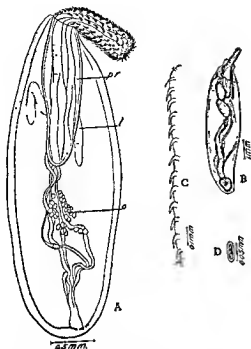
FIG. 34.25 — *Plagiorhynchus formosus*. (A) Young female: l, lemniscus; a, ovary, pr, proboscis receptacle (from Janes 1928). (B) Male. (C) Hooks from proboscis. (D) Egg. (Enlarged.) (From Van Cleave, 1918.)

FIG. 34.26 — *Polymorphus boschadis*. (A) Male. (B) Larva from *Gammarus pulex*. (C) Proboscis of larvae. (Enlarged.) (From Lühe, 1911.)

(1928) from the small intestine of a chicken collected at Vineland, New Jersey. Other bird hosts from which this species has been reported are the flicker, collected at Bowie, Maryland, the crow, collected at Washington, D.C.; and the robin in New Jersey (Fig. 34.25A, B, C, and D).

Polymorphus boschadis (Schrank, 1788)

Wickware (1922) reported *Polymorphus boschadis* (Schrank, 1788) from the duck

in Canada. This parasite is reported as causing serious injury and death among domesticated waterfowl, especially in young birds. It causes an inflammation of the intestine with subsequent anemia and cachexia. According to Schlegel (1921), the birds are sick only a short time, the gait is staggering, and the head and wings droop (Fig. 34.26A, B, and C).

REFERENCES

Ackert, J. E.: 1931 The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). Parasit. 23 360.
——: 1940. The large roundworm of chickens. Vet. Med. 35:106.
—— and Beach, T. D.: 1933. Resistance of chickens to the nematode, *Ascaridia lineata*, affected by dietary supplements. Trans. Am. Micr. Soc. 52:51.
——, Edgar, S. A., and Frick, L. P.: 1939. Goblet cells and age resistance of animals to parasitism. Trans. Am. Micr. Soc. 58.81
——, Eisenbrandt, L. L., Wilmoth, J H., Glading, B., and Pratt, I.: 1935. Comparative resistance of five breeds of chickens to the nematode *Ascaridia lineata* (Schneider). Jour. Agr. Res. 50.607.
—— and Graham, G. L.: 1935 The efficacy of carbon tetrachloride in roundworm control. Poultry Sci. 14:228.
Alicata, J. E.: 1938. Studies on poultry parasites. Rep. Hawaii Agr. Exper. Sta. (1937).93.
——: 1940. Poultry parasites. Rep. Hawaii Agr. Exper. Sta. (1939).66

Allen, A. A.: 1925. The grouse disease in 1924. Bul. Am. Game Protect. Assn. 14:11, 12, 20.
——— and Gross, A. O.: 1926. Report of the ruffed grouse investigations; season of 1925–26. Am. Game 15:81, 86.

Allen, R. W., and Wehr, E. E.: 1942. Earthworms as possible intermediate hosts of Capillaria caudinflata of the chicken and poultry. Proc. Helminth. Soc. Wash. 9:72.

Andrews, J. S., Taylor, A. L., and Swanson, L. E.: 1943. Fumigation of soil with methyl bromide as a means of destroying infective stages and intermediate hosts of some internal parasites of mammals. Proc. Helminth. Soc. Wash. 10:4.

Baker, A. D.: 1910. The internal parasites of poultry in Quebec. Scient. Agr. 11:150.

Barber, L. B.: 1916. Live stock disease investigations. Ann. Rep., Guam Agr. Exper. Sta. (1915):25.

Banle, C.: 1912. Sur une espèce de trichostrongyle signalée chez le dindon (Meleagris gallopavo domestica (L). Bul. Soc. zool. (France) 37:126.

Bradley, R. E.: 1955. Observations on the anthelmintic effect of piperazine citrate in chickens. Vet. Med. 50:444.

Bump, G.: 1935. Ruffed grouse in New York State during the period of maximum abundance. Trans. 21st Am. Game Conf.:364.

Bunyea, H., and Creech, G. T.: 1926. The pathological significance of gizzard-worm disease of geese. No. Am. Vet. 7:47.

Chitwood, B. G., and Chitwood, M. B.: 1937. An Introduction to Nematology. Monumental Publishing Company, Baltimore, Maryland. Section 1, Part 1.

Clapham, P. A.: 1934. Experimental studies on the transmission of gapeworm (Syngamus trachea) by earthworms. Proc. Roy. Soc, London, series B, 115:18.

——— 1950. On sterilizing land against poultry parasites. Jour. Helminth. 24:137.

Clarke, M. L.: 1962. Capillariasis in poultry. Vet. Rec. 74:1451.

Colglazier, M. L., Foster, A. O., Enzie, F. D., and Thompson, D. E.: 1960. The anthelmintic action of phenothiazine and piperazine against Heterakis gallinae and Ascaridia galli in chickens. Jour. Parasitol. 46:267.

Cram, E. B.: 1926a. A parasitic nematode as the cause of losses among domestic geese. No. Am. Vet. 7:27.

——— 1926b. Subulura brumpti from the turkey in Puerto Rico. Jour. Parasit. 12:164.

——— 1926c. A parasitic disease of the esophagus of turkeys. No. Am. Vet. 7:46.

——— 1926d. New records of economically important nematodes in birds. Jour. Parasit. 12:113.

——— 1927. New records of distribution for various nematodes. Jour. Parasit. 14:70.

——— 1928. Nematodes of pathological significance found in some economically important birds in North America. U.S.D.A., Tech. Bul. 49:1.

——— 1929. A new roundworm parasite, Strongyloides avium, of the chicken, with observations on its life history and pathogenicity. No. Am. Vet. 10:27.

——— 1931a. Internal parasites and parasitic diseases of the bobwhite. Nematodes (roundworms) in quail. In Stoddard, H. L.: The Bobwhite Quail; Its Habits, Preservation, and Increase. Charles Scribner's Sons, New York, pp. 240–96.

——— 1931b. Developmental stages of some nematodes of the Spiruroidea parasitic in poultry and game birds. U.S.D.A., Tech. Bul. 227.

——— 1936a. Species of Capillaria parasitic in the upper digestive tract of birds. U.S.D.A., Tech. Bul. 516:1.

——— 1936b. Notes concerning internal parasites of poultry in Puerto Rico. Agr. Notes 70, Puerto Rico Agr. Exper. Sta., U.S.D.A., May 15, five mimeographed leaves.

——— and Wehr, E. E.: 1934. The status of species of Trichostrongylus of birds. Parasit. 26:355.

Crawford, M.: 1940. Infection of adult fowls with Syngamus tracheaelis. Indian Jour. Vet. Sci. and Anim. Husb. 10:293.

Cuckler, A. C., and Alicata, J. E.: 1944. The life history of Subulura brumpti, a cecal nematode of poultry in Hawaii. Trans. Am. Micr. Soc. 63:345.

Davis, D. E.: 1940. Nicotine in the control of Ascaridia lineata in fowls. Vet. Med. 35:109.

Dalmans, C.: 1929. Report of the parasitologist. Rep. Puerto Rico Agr. Exper. Sta. (1927):27.

Emmel, M. W.: 1939. Observations on Capillaria contorta in turkeys. Jour. Am. Vet. Med. Assn. 94:612.

Farr, M. M.: 1956. Survival of the protozoan parasite Histomonas meleagridis, in feces of infected birds. Cornell Vet. 46:178.

Foster, A. O.: 1939. Some helminthic parasites recovered from domesticated animals (excluding equines) in Panama. Proc. Helminth. Soc. Wash. 6:101.

Fraser, M. N.: 1962. Artificial propagation of Capillaria obsignata in chickens. Poultry Sci. 41:551.

Freeborn, S. B.: 1923. The control of the suckered roundworms of poultry. Cornell Vet. 13:223.

Gibbs, B. J.: 1962. The occurrence of the protozoan parasite Histomonas meleagridis in the adults and eggs of the cecal worm Heterakis gallinae. Jour. Protozool. 9:288.

Goble, F. C., and Kutz, H. L.: 1945. The genus Dispharynx (Nematoda: Acuariidae) in galliform and passeriform birds. Jour. Parasit. 31:323.

Graham, R., Thorp, F., and Hectorne, R. L.· 1929. Capillaria in chickens. Jour. Am. Vet. Med. Assn. 74:1060.

Graybill, H. W.: 1924. Capillaria columbae (Rud) from the chicken and turkey. Jour. Parasit. 10:205.

———, and Smith, T.: 1920. Production of fatal blackhead in turkeys by feeding embryonated eggs of Heterakis papillosa. Jour. Exper. Med. 31:647.

Griffiths, H. J., Leary, R. M., and Fenstermacher, R.· 1954. A new record for gapeworm (Cyathostoma bronchialis) infection of domestic geese in North Amexies. Am. Jour. Vet. Res. 15:298.

Guthrie, J. E., and Harwood, P. D.: 1942. The efficacy of phenothiazine and nicotine-bentonite for the removal of Heterakis gallinae and Ascaridia galli from chickens. Jour. Parasit. 28 (Suppl).21.

Hall, N. C., and Shillinger, J. E.· 1925a Miscellaneous tests of carbon tettachloride as an anthelmintic. Jour. Agr. Res. 23:163.

———, and Shillinger, J. E.: 1925b. The removal of heterakids from the ceca of chickens by rectal injections of anthelmintics. Jour. Am. Vet. Med. Assn 62:623.

Harwood, P. D., and Stunz, D. E.: 1945. Phenothiazine and nicotine-bentonite as an anthelmintic in turkeys. Proc. Helminth. Soc. Wash. 12:1.

Herms, W. B., and Beach, J. R.: 1916. Round worms in poultry—life history and control. Calif. Agr. Expet. Sta., Circ. 150

Horton-Smith, C., and Long, P. L.· 1956. The anthelmintic effect of three piperazine derivatives on Ascaridia galli (Schrank, 1788) Poultry Sci. 35:606.

Hung, S. L.: 1926 Pathological lesions caused by Capillaria annulata. No. Am. Vet. 7:49.

Itagaki, S.· 1927. On the life history of the chicken nematode, Ascaridia perspicillum. Proc. Third World's Poultry Cong., p. 339

Jones, M.· 1928 An acanthocephalid, Plagiorhynchus formosus, from the chicken and robin. Jour. Agr. Res. 36:773.

Kattman, L., Tanada, Y, Holdaway, F. G., and Aliesta, J. E.: 1950. Laboratory tests to determine the efficacy of certain insecticides in the control of arthropods inhabiting poultry manure Poultry Sci 29:336

Kaupp, B. F 1909 Echinorhynchus canis Am. Vet. Rev. 35:154

Kelley, G W 1962. Removal of Syngamus trachea, gapeworm, from pheasants with subcutaneously injected Disophenol. Poultry Sci. 41:1358.

Kendall, S. B. 1959 The occurrence of Histomonas meleagridis in Heterakis gallinae. Parasit. 49 169

Knapp, S. E., and Hansen, M. F.· 1960. Efficacy of carbon bisulphide against Ascaridia galli (roundworm) of chickens. Poultry Sci. 39:1105.

Komarov, A. and Beaudette, F. R· 1931. Ornithostrongylus quadriradiatus in squabs. Jour. Am. Vet. Med. Assn. 79:333.

Le Roux, P. L· 1926 Helminths collected from the domestic fowl (Gallus domesticus) and the domestic pigeon (Columba livia) in Natal. 11th–12th Rep. Director Vet. Educ. and Res., Dept. Agr. Union South Africa, Pretoria, pt. 1, Sept.:209.

——· 1930 Helminthiasis of domestic stock in the Union of South Africa. Jour. South African Vet. Med. Assn 1 43 (Oct).

Levine, P. P.: 1936 The treatment of ascariasis in chickens. Cornell Vet. 26:120.

——· 1938 Infection of the chicken with Capillaria columbae (Rud.). Jour. Parasit. 24:45.

McCulloch, E. C. and Nicholson, L. G 1940. Phenothiazine for the removal of Heterakis gallinae from chickens Vet. Med. 35 398

Madsen, H.: 1945 The species of Capillaria parasitic in the digestive tract of Danish gallinaceous and anatine game birds. Danish Rev. Game Biol. 1:1.

——· 1951 Notes on the species of Capillaria Zeder. 1800 known from gallinaceous birds. Jour. Parasit. 37:257.

Morehouse, N. F 1944 Life cycle of Capillaria caudinflata, a nematode parasite of the common fowl. Iowa State Coll. Jour. Sci 18(2), Jan., p. 217.

Oliver, L. J.· 1937 The occurrence of Syngamus trachea in mature chickens. Proc. Helminth. Soc. Wash. 10 87

Ortlepp, R. J.: 1923 The life history of Syngamus trachealis (Montagu) v. Siebold, the gapeworm of chickens. Jour Helminth. 1:119

Pande, B. P., Rai, P., and Srivataua, J. S· 1963 A note on some pathogenic effects observed in certain nematode infections of wild aquatic birds with remarks on its significance. Poultry Sci. 39.1121.

Price, E. W.· 1929. Acanthocephalid larvae from the esophagus of turkey poults. Jour. Parasit. 15 290.

Ransom, B. H.: 1921. The turkey an important factor in the spread of gapeworms. U.S.D.A., Bul. 939.

Riedel, B. B.: 1951. Group treatment with Caricide for ascariasis in poultry. Jour. Parasit. 37.31R.

Riley, W. A., and James, L· 1922. Life history and methods of control of the chicken nematode (Heterakis papillosa, Bloch). 30th Ann. Rep. Minn Agr. Exper. Sta. (1921–22) p. 70.

Roberts, F. H. S.: 1937. Studies on the life history and economic importance of *Heterakis gallinae* (Gmelin, 1790; Freeborn, 1923), the caecum worm of fowls. Australian Jour. Exper. Biol. and Med. Sci. 15:429.

Sanders, D. A.: 1929. Manson's eyeworm of poultry. Fla. Agr. Exper. Sta., Bul. 206:565.

Schlegel, M.: 1921. *Echinorhynchus polymorphus* Brems., seuchenhaftes Entensterben verursachend. Arch. wiss. u. prakt. Tierheilk. 47:216.

Schwabe, C. W.: 1951. Studies on *Oxyspirura mansoni*, the tropical eyeworm of poultry. II. Life history. Pacific Sci. 5:18.

Shillinger, J. E.: 1942. Diseases of farm-raised game birds. U.S.D.A. Yearbook:1230.

Shumard, R. F., and Eveleth, D. F.: 1955. A preliminary report on the anthelmintic action of piperazine citrate on *Ascaridia galli* and *Heterakis gallinae* in hens. Vet. Med. 50:203.

Stevenson, E. C.: 1904. A new parasite (*Strongylus quadriradiatus* n. sp.) found in the pigeon. Bur. Anim. Ind., U.S.D.A., Circ. 47:1.

Stoddard, H. L.: 1931. The Bobwhite Quail; Its Habits, Preservation, and Increase. Charles Scribner's Sons, New York.

Sugimoto, M., and Nishiyama, S.: 1937. On the nematode, *Tropisurus fissispinus* (Diesing, 1861), and its transmission to chickens in Formosa. Jour. Jap. Soc. Vet. Sci. 16:305.

Swales, W. E.: 1933. *Tetrameres crami* sp. nov., a nematode parasitizing the proventriculus of a domestic duck in Canada. Canad. Jour. Res. 8:334.

Tanada, Y., Holdaway, F. G., and Quisenberry, J. H.: 1950. DDT to control flies breeding in poultry manure. Jour. Econ. Entom. 46:50.

Taylor, E. L.: 1938. An extension to the known longevity of gapeworm infection in earthworms and snails. Vet. Jour. 94:527.

Thienpont, D., and Mortelmans, J.: 1962. Methyridine in the control of intestinal capillariatis in birds. Vet. Rec. 74:8550.

Tugwell, R. L., and Ackert, J. E.: 1952. On the tissue phase of the life cycle of the fowl nematode *Ascaridia galli* (Schrank). Jour. Parasit. 38:277.

Tyzzer, E. E.: 1926. *Heterakis vesicularis* Frölich, 1791; a vector of an infectious disease. Proc. Soc. Exper. Biol. and Med. 23:708.

———: 1928. Entero-hepatitis in turkeys and its transmission through the agency of *Heterakis vesicularis*. Proc. Third World's Poultry Cong., p. 286.

Unbe, G.: 1922. Observations on the development of *Heterakis papillosa* Bloch in the chicken. Jour. Parasit. 8:167.

Van Volkenberg, H. L.: 1938. Check list of parasites found among principal domestic animals in Puerto Rico. Proc. Helminth. Soc. Wash. 5:7.

Venard, C.: 1933. Helminths and coccidia from Ohio bobwhite. Jour. Parasit. 19:205.

Vunella, G., and Vicenzoni, V.: 1955. L'azione antelmintica dell'adipato di piperazina sugli ascaridi de pollo. Clin. Vet. 78:565.

Viguera, P.: 1929. Una enfermedad parasitaria epizootica de las palomas. Agr. y. zootec. 8:157.

———: 1931. Nota sobre algunos helmintos de *Meleagris gallopavo*, encontrados en Cuba, con descripcion de una nueva especie. Habana, Cuba. (2) pp.

Walker, H. D.: 1886. The gapeworm of fowls (*Syngamus trachealis*): The earthworm (*Lumbricus terrestris*), its original host. Also, on the prevention of the disease in fowls called the gapes, which is caused by this parasite. Bull. Buffalo Soc. Nat. Sci. 5:47.

Ward, J. W.: 1945. A new locality record for five species of helminth parasites of the bobwhite quail. Proc. Helminth. Soc. Wash. 12:71.

Wehr, E. E.: 1936. Earthworms as transmitters of *Capillaria annulata*, the "cropworm" of chickens. No. Am. Vet. 17:18.

———: 1937a. Relative abundance of crop worms in turkeys. Macroscopic differentiation of species. Vet. Med. 32:230.

———: 1937b. Observations on the development of the poultry gapeworm, *Syngamus trachea*. Trans. Am. Micr. Soc. 56:72.

———: 1939a. Studies on the development of the pigeon capillarid, *Capillaria columbae*. U.S.D.A., Tech. Bul. 679:19.

———: 1939b. Domestic fowls as hosts of the poultry gapeworm. Poultry Sci. 18:432.

———, and Allen, R. W.: 1945. Additional studies on the life cycle of *Capillaria caudinflata*, a nematode parasite of chickens and turkeys. Proc. Helminth. Soc. Wash. 12:12.

———, Harwood, P. D., and Schaffer, J. M.: 1939. Barium antimonyl tartrate as a remedy for the removal of gapeworms from chickens. Poultry Sci. 18:63.

Whitney, L. F.: 1957. Practical test of the efficacy of piperazine citrate in pigeons. Vet. Med. 52:258.

Wickware, A. B.: 1922. Notes on the parasites of domesticated fowls in Canada. Canad. Vet. Record 3:142.

Wilcox, E. V., and McClelland, C. K.: 1915. Eyeworm of chickens. Hawaii Agr. Exper. Sta., Bul. 43:1.

Yeatter, R. E.: 1934. The Hungarian partridge in the Great Lakes region. Univ. Michigan, School Forestry and Conserv., Bul. 5:92.

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35

Cestodes of Poultry

The cestodes or tapeworms are flattened, ribbon shaped, usually segmented worms. As adults, they are found principally in the intestines of their hosts. These worms are hermaphroditic and lack both a mouth and an alimentary canal.

The class CESTODA has recently been subdivided into fourteen orders (Wardle and McLeod, 1952). The tapeworms which occur in poultry of this country have been grouped by these authors into one order, namely CYCLOPHYLLIDEA. The CYCLOPHYLLIDEA, or taenioid cestodes are, as adults, parasitic chiefly in the higher vertebrates and are of considerable economic and medical importance. These tapeworms are characterized by having a scolex with four cup-shaped suckers and with or without a rostellum.

The taenioid cestodes are grouped into a number of families, four of which contain species infecting poultry. The worms of the family Anoplocephalidae possess neither rostellum nor hooks. The proglot-

tids are usually wider than long, and each proglottid contains one or two sets of genital organs. The genital pores are marginal, and the eggs frequently contain "pyri-form" bodies. The species *Aporina dela-fondi* belongs to this family. The family Davaineidae is composed of tapeworms having a scolex with a simple rostellum which is armed with one or more rows of numerous hammer-shaped hooks. The suckers are usually also provided with hooks. Each proglottid contains one or two sets of genital organs. The uterus is persistent and saclike, or replaced by either numerous egg capsules or a paruterine body which later becomes transformed into a single egg capsule. Poultry tapeworms of the genera Davainea and Raillietina belong to this family. In the family Dilepi-didae, the rostellum is usually armed, but the suckers are unarmed. The genital pores are marginal, one or two in each segment. The uterus is saclike, or resolved into egg capsules — uterus with or without

paruterine body. The poultry tapeworms, *Amoebotaenia sphenoides* and *Choanotaenia infundibulum*, belong to this family. The Hymenolepididae is characterized by having a scolex with rostellum usually armed with a single row of hooks; the suckers are unarmed. The genital pores are usually unilateral, rarely two in each segment. The uterus is usually persistent and saclike. The eggs are enclosed in three envelopes. The species of poultry tapeworms belonging to the genus Hymenolepis belong to this family.

General morphology. Structurally, a complete tapeworm consists of a head, neck or growth zone, and a variable number of segments or divisions. The head or scolex of a taeniid tapeworm consists of four cuplike organs or suckers which may surround a terminal retractile organ known as the rostellum. Hooks may or may not be found on the rostellum, and deciduous spines often arm the suckers. The number, size, and shape of the rostellar hooks vary as to species, and these variations are used by systematists in differentiating species and even genera of tapeworms. The term "neck" or "growth zone" is applied to the narrowed and unsegmented region located just back of the head and in some cestodes, is not macroscopically distinct from the head. The segments or divisions of a tapeworm when taken collectively are generally spoken of as the strobila, and each segment or division as a proglottid. The size, shape, and development of proglottids vary tremendously even in the same individual worm. The anterior segments are usually broader than long and contain few, if any, recognizable internal organs. Those segments near the middle of the body of the tapeworm may have the antero-posterior diameter proportionately greater than that of the anterior segments. These segments are spoken of as mature segments, since in these proglottids both the male and female reproductive organs are distinctly differentiated. Eggs are not usually found in segments of this part of the body. The terminal or gravid segments are variable

in shape and usually contain the uteri and eggs or only eggs with the uterus either partly or wholly obliterated.

Since tapeworms lack an alimentary canal, food is absorbed through the surface of the body.

A tapeworm grows from the neck backwards, and segments are continually being budded off from the proliferating tissue found in this region. Therefore, the segments farthest removed from the growing region are the oldest from the standpoint of development. The newly formed segment contains no distinguishable organs, while the terminal segments of a completely formed tapeworm may be nothing more than egg sacs. The latter are known as gravid segments and are the ones usually found in the droppings of infected birds.

All adult tapeworms of poultry are found usually in the small intestines of their hosts. However, *Hymenolepis megalops*, the large-headed tapeworm of ducks, occurs in the cloaca and bursa Fabricius of these birds. Each species of tapeworm usually shows some predilection for a particular portion of the small intestine to which to attach. The species *Hymenolepis carioca*, *H. cantaniana*, *Amoebotaenia sphenoides*, and *Davainea proglottina* are usually found in the duodenal region of the small intestine; *Raillietina cesticillus*, *Choanotaenia infundibulum*, and *Metroliasthes lucida* in the jejunal region; and *Raillietina tetragona* and *R. echinobothrida* in the distal portion or ileum. However, in heavy infections, tapeworms may be found in portions of the small intestine other than their more normal locations.

Adult tapeworms of poultry differ considerably as to length and as to number of proglottids or segments. *Davainea proglottina* and *Amoebotaenia sphenoides* are two of the smallest tapeworms found in poultry. Mature specimens of the former species measure up to 4 mm. in length and have a range of segments from 4 to 9, while those of the latter species reach a length of from 2 to 3.5 mm. and possess approximately 30 proglottids.

Raillietina echinobothrida and *R. tetragona*, on the other hand, are two of the largest tapeworms infecting poultry. Mature specimens of both of these tapeworms may attain a length of approximately 25 cm. and possess large numbers of proglottids.

Development. In the case of every tapeworm of poultry in which the life history is known, an intermediate host is necessary for the completion of the life cycle. Investigations have shown invariably that intermediate hosts of tapeworms have always been found to be invertebrates, such as a beetle, fly, snail, slug, or crustacean. Tapeworm segments are devoured by dung-feeding insects either along with their normal food or because they are attracted to the attention of the invertebrates by their movements.

The type of intermediate host that serves a particular tapeworm in its successful transference from one bird host to another depends to a large degree on the habits of the avian host. In the case of terrestrial birds, such as chickens, turkeys, guinea fowls, etc., which deposit their body wastes principally away from ponds and streams, the intermediate hosts must necessarily have to be forms of animal life that lead a terrestrial life, or at least an amphibious one. On the other hand, tapeworms inhabiting water birds, such as ducks and geese, usually have aquatic invertebrates as natural intermediate hosts.

Invertebrates, which serve as intermediate hosts of poultry tapeworms, become infected with larval tapeworms by ingesting, along with their food, the free eggs or the egg-bearing segments voided by the infected birds. Following ingestion the eggs hatch in the digestive tract, the embryos or larvae penetrate the intestinal wall, enter the body cavity, and after a few days become transformed into small, white, bladderlike, spherical bodies, known as cysticercoids (small cysts). These cysts are distinctly visible to the unaided eye when placed in water after removal from the body of the intermediate host. Under proper magnification the head of

the tapeworm may be seen near the center of the cyst.

Approximately 3 weeks are required for the embryos to develop into the cysticercoid stage after the eggs have been ingested by the intermediate host. No further development of the tapeworm takes place in the invertebrate host. The cysticercoids may remain alive in the invertebrate host and infective to the bird host for many months.

Poultry become infected with tapeworms by swallowing, with their food and water, insects, snails, slugs, and other forms of animal life that may serve as intermediate hosts of these parasites. The cysticercoid is freed from the body of the intermediate host by the action of the digestive juices. Soon after the cysticercoid is liberated, the head evaginates and becomes attached to the intestinal wall. New segments or proglottids begin to form immediately at the neck region, and within approximately 3 weeks a mature tapeworm is formed. The entire life cycle, therefore, takes about 6 weeks for completion, but under unfavorable conditions a longer period of time may be necessary.

Gross pathology. A few tapeworms may produce little or no perceptible gross pathological changes in the intestines. However, in heavy tapeworm infections, a more or less extensive catarrhal enteritis and diarrhea may result. At least one species of tapeworm, *Raillietina echinobothrida*, causes the formation of nodules in the intestinal wall. Inasmuch as this condition closely resembles tuberculosis, it is important that the two conditions be kept in mind in attempting to arrive at a diagnosis of the condition present. The presence of intestinal nodules — sometimes distinctly visible on the outer surface of the intestinal wall — and the absence of tapeworms is strongly suggestive of tuberculosis. However, a diagnosis of intestinal tuberculosis should not be made without first eliminating tapeworms of this species as a cause of the nodules. Mature specimens of this tapeworm are usually several centimeters long, but observations

have shown that, in many cases, infections involved individual tapeworms containing only a very few segments, sometimes only the heads. In such cases, the parasites may be easily overlooked if only a casual or hurried examination is made. In doubtful cases, the intestine should be scraped with a scalpel or other suitable instrument and a careful examination made of the scrapings under suitable magnification for the presence of small tapeworms or their heads. The presence of tapeworms and the absence of tubercles in the liver and other organs are indicative of tapeworm disease. Another species of tapeworm, *Raillietina tetragona*, which is morphologically very similar to *R. echinobothrida* and often confused with it, has not been definitely associated with tuberculosislike lesions.

Leg weakness and paralysis have frequently been attributable to tapeworm infection. However, the relationship of tapeworms to these diseases is still unknown. Should these conditions be intimately associated with the presence of tapeworms, the mere removal of the parasite should clear up the condition. The fact that birds which had previously shown symptoms of leg weakness and paralysis were free from tapeworms at necropsy seems to disprove the idea that tapeworms are in a large degree responsible for these conditions. Capillary congestion; lymphocyte, polymorphonuclear, and eosinophil infiltration; proliferation of epithelium; and fibrosis are other conditions which have been associated with tapeworm infections.

There is some evidence to show that birds heavily parasitized with tapeworms are not as productive as uninfected ones. Under ordinary conditions birds may tolerate a fairly heavy tapeworm infec-

tion, at least for a time. However, young birds and hens in heavy production do not fare so well when heavily infected with these parasites.

Importance of cestodes as parasites of poultry. Chickens in this country may be infected with one to as many as seven species of tapeworms. With the exception of one or two of these species, all are of common occurrence. A few years ago, the list of intermediate hosts of poultry tapeworms was small, but investigations within the last few years have been responsible for an alarming increase in the number of invertebrate intermediate hosts that tapeworms of poultry may utilize for the development of their larvae.

The control of poultry tapeworms involves treatment for the removal of the tapeworms themselves and the reduction of the numbers of intermediate hosts by sanitary measures. Since the treatment of fowl taeniasis is still in an unsatisfactory state, sanitation has almost wholly been relied upon to prevent tapeworm infection. This method of control involves, first of all, the proper disposal of poultry manure containing the eggs of tapeworms so that the intermediate hosts cannot become infected with the larval stages of these parasites. Many of the intermediate hosts are flying insects, and once the latter have become infected with larval tapeworms they may be responsible for the spread of the disease to distant flocks. Recent investigations have indicated that clean birds held in close proximity to infected birds will invariably become infected with tapeworms within a relatively short time.

The species of cestodes parasitizing poultry of the United States belong to four families which may be differentiated by the following key:

1. Head armed with numerous hammer-shaped hooks . . . . . Davaineidae
   Head armed with hooks not hammer-shaped, or unarmed 2
2. Testes few, 1 to 4, rarely more . . . . . Hymenolepididae
   Testes numerous, more than 4 3
3. Head lacking rostellum; no paruterine organs in species occurring in poultry . . . . . Anoplocephalidae
   Head with retractile rostellum, usually armed, or, rarely, unarmed; rarely without rostellum; with or without paruterine organs . . . . . Dilepididae

LIST OF TAPEWORMS KNOWN FROM POULTRY OF UNITED STATES

The following is a list of the species of tapeworms found in poultry of this coun-try, with their primary and secondary hosts, usual location and kinds of poultry affected.

Tapeworms	Location	Intermediate hosts	Definitive hosts
Davainea proglottina	Duodenum	Slugs, snails	Chicken
Davainea meleagridis	Duodenum	Unknown	Turkey
Amoebotaenia cuneata	Duodenum	Earthworms	Chicken, Turkey
Hymenolepis carioca	Duodenum	Stable fly Dung beetles	Chicken, Turkey Bobwhite quail
Hymenolepis cantaniana	Duodenum	Beetles	Chicken, Turkey Peafowl Bobwhite quail
Raillietina cesticillus	Jejunum	Housefly Beetles	Chicken, Turkey Guinea fowl Bobwhite quail Gray jungle fowl
Choanotaenia infundibulum	Jejunum	Housefly, Beetles	Chicken, Turkey
Raillietina tetragona	Ileum	Ants	Chicken, Turkey Guinea fowl, Peafowl Bobwhite quail
Raillietina echinobothrida	Ileum	Ants	Chicken, Turkey
Metroliasthes lucida	Ileum	Grasshoppers	Turkey, Chicken Guinea fowl
Hymenolepis compressa	Intestine	Unknown	Duck, Goose
Hymenolepis introversa	Intestine	Unknown	Duck
Hymenolepis megalops	Cloaca and bursa of Fabricius	Unknown	Duck
Hymenolepis tritesticulata	Intestine	Unknown	Duck
Hymenolepis coronula	Small Intestine	Crustaceans Snails	Duck
Hymenolepis lanceolata	Small Intestine	Crustaceans	Goose
Hymenolepis tenuirostris	Small Intestine	Crustaceans Crayfish	Duck, Goose
Raillietina magninumida	Small Intestine	Beetles	Guinea fowl
Raillietina ransomi	Small Intestine	Unknown	Wild turkey
Raillietina williamsi	Small Intestine	Unknown	Wild turkey
Raillietina georgiensis	Small Intestine	Ants	Wild turkey Domestic turkey
Aporina delafondi	Small Intestine	Unknown	Pigeon
Fimbriaria fasciolaris	Small Intestine	Water flea	Chicken

CLASSIFICATION OF POULTRY TAPEWORMS

The tapeworms of poultry belong to the general group designated as taenioid cestodes, which are characterized primarily by the presence of four cup-shaped suckers upon the head. The following key will aid in the differentiation of the genera of tapeworms found in poultry of this country:

1.	Rostellum absent	2
	Rostellum present	3
2.	Paruterine organ present	*Metroliasthes*
	Paruterine organ absent	*Aporina*
3.	Mature worms small, usually not longer than 4 to 5 mm.	1
	Mature worms large, longer than above . . .	5
4.	Strobila consisting of 2 to 9 segments . . .	*Davainea*
	Strobila consisting of numerous segments . . .	*Amoebotaenia*
5.	Testes 3 in number	6
	Testes more than 3 in number	7
6.	With a well-developed pseudo-holdfast organ, in addition to a small, true holdfast organ, containing no genital primordia . . . *Fimbriaria*
	With only a true holdfast organ . . . . . *Hymenolepis*
7.	Rostellum armed with a single row of 16 to 20 hooks, each 20 to 30μ long . . . . *Choanotaenia*
	Rostellum armed with either a single row or double row of 100 or more hooks, each 6 to 15μ long . . . . . *Raillietina*

DESCRIPTIONS OF POULTRY TAPEWORMS

To facilitate somewhat the identification of the species of poultry tapeworms, they have been grouped according to their normal location within the intestine of the hosts, i.e., duodenum, jejunum, and ileum, with a brief description of each species.

Five species of tapeworms normally inhabit the duodenal region. Three of these species, *Davainea proglottina*, *D. meleagridis*, and *Amoebotaenia cuneata*, are very small worms, rarely exceeding 5 mm. in length, and possessing relatively few segments. The other species, *Hymenolepis carioca* and *H. cantaniana*, are relatively long worms and are composed of many segments.

Dilepididae

Members of this family are characterized by having a single set of reproductive organs in each proglottid. The uterus is sac-like and more or less lobed or reticulate. Paruterine bodies are present or absent.

Amoebotaenia cuneata (Linstow, 1872)

Synonyms. *Taenia cuneata* von Linstow, 1872, not Batsch, 1786; *Taenia sphenoides* Railliet, 1892; *Dicranotaenia sphenoides* (Railliet, 1892) Railliet, 1896.

Description. Mature worms 2 to 3.5 mm. long, triangular or roughly fusiform in shape (Fig. 35.1A). Suckers unarmed; rostellum armed with a single row of 12 to 14 hooks, 25 to 32μ long (Fig. 35.1B). Genital pores usually regularly alternate, located at extreme anterior point of segment margin. Testes 12 to 15 in number, usually in a transverse row across posterior part of segment. Eggs (Fig. 35.1C) not contained in capsules.

This tapeworm, which usually occurs in the duodenal region of the small intestine, is apparently not a common parasite of poultry in the United States. It has been reported from chickens in Kansas by
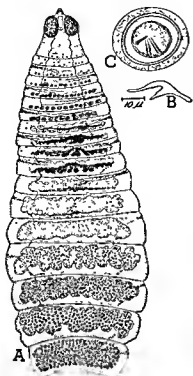
FIG. 35.1.— Amoebotaenia cuneata. (A) Entire worm. (From Mönnig, 1926.) (B) Rostellar hook. (C) Egg. Original.

Ferry (1934), from chickens in Texas by Adams and Ceiser (1933), from chickens in Tennessee by Todd (1946), from chickens and turkeys in Michigan by Stafseth (1940), and from chickens in Alabama by Edgar (1956).

Life history. The intermediate host of this tapeworm is the earthworm. The earthworms *Eisenia (Helodrilus) foetida*, *Pheretina pequana*, *Ocnerodrilus (Ilyogenia) africanus*, and *Allolobophora chlorotica* have been found to serve as intermediate hosts of this tapeworm. Mönnig (1927) grew the cysticercoids in earthworms (*Ocnerodrilus (Ilyogenia) africanus*) in 14 days. Four weeks were required for the cysticercoids to develop into adult tapeworms in chickens. Cysticercoids from earthworms were identified as this species by Grassi and Rovelli (1889) and Meggitt (1916). Chickens be-

come infected by eating earthworms which carry the infective larva or cysticercoids of this cestode parasite.

Pathology. The damage done by this tapeworm is comparatively slight, according to Meggitt (1926). However, deaths in poultry as being due to this parasite have been reported.

Choanotaenia infundibulum (Bloch, 1779)

Synonyms. *Taenia infundibulum* Bloch, 1779; *Drepanidotaenia infundibuliformis* (Goeze, 1782) Railliet, 1893; *Choanotaenia infundibuliformis* (Goeze, 1782) Railliet, 1896.

Description. Mature worms attain a length of 23 cm. Suckers unarmed (Fig. 35.2A); rostellum armed with a single row of 16 to 20 hooks, occasionally 22, 20 to 50μ long (Fig. 35.2B). Genital pores irregularly alternate. Testes 25 to 40, occasionally as many as 55 to 60, grouped in posterior part of segment (Fig. 35.2C). Eggs with elongated filaments, not contained in capsules (Fig. 35.2D).

This species may be readily distinguished from the other poultry tapeworms by the rostellum, which is armed with a single row of relatively few and very large hooks, and bipolar egg filaments.

This cestode inhabits principally the jejunal region of the small intestine of chickens and turkeys and is widely distributed among these birds in the United States.

Life history. Birds become infected with adults of *C. infundibulum* by eating house flies, grasshoppers, and several species of beetles. Cysticercoids have been found in house flies and in some species of beetles as natural infections, and also after the insects have been fed eggs of this tapeworm. Horsfall and Jones (1937) reported that at a temperature of 75°–90° F., 17 to 20 days is the minimum time for development of the cysticercoids to the infective stage in the grasshopper, *Melanoplus femurrubrum*. At a temperature of 60°–75° F., 48 days is the minimum time for the development of the cysticercoids in
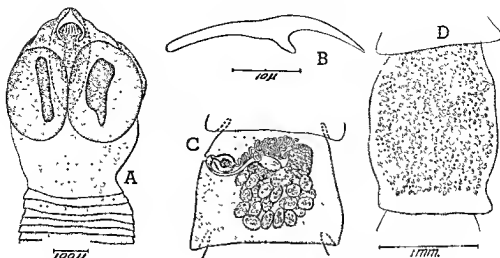
FIG. 35.2 — *Choanotaenia infundibulum.* (A) Scolex. (B) Rostellar hook. (C) Mature segment. (D) Gravid segment. (From Ransom, 1905.)

the beetle, *Aphodius granarius.* The adult worm in the chicken requires from 2 to 3 weeks for development to maturity.

Pathology. Probably similar to *R. cesticillus.*

Metroliasthes lucida Ransom, 1900

Description. Mature worms about 20 cm. long. Suckers unarmed, rostellum lacking (Fig. 35.3A). Genital pores irregularly alternate, near middle of, or in gravid segments, definitely posterior to middle of segment margin. Uterus, when fully developed, consisting of two sacs, lying side by side and very close together in posterior part of segment (Fig. 35.3D). Paruterine organ, a conical structure, developing anterior to uterus, eventually becoming a heavy-walled egg capsule for the retention of the eggs (Fig. 35.3E).

This species is a very common tapeworm of turkeys in this country. It was reported from a chicken by Ransom (1905), but he evidently doubted the validity of the host record since he stated that the occurrence of *Metroliasthes lucida* in chickens is doubtful. However, the occurrence of this species in chickens has been reported more recently by Rietz (1930) from West Virginia, by Southwell (1921) from India, and by Schwartz (1925)

from South Africa. It is readily recognized by the large unarmed head and the prominent spherical egg capsule, which is easily seen in the posterior part of each of the transparent segments in the posterior part of the body.

Life history. Cysticercoids were obtained by Jones (1930b) from grasshoppers several weeks after feeding to the insects gravid segments of *M. lucida;* both laboratory-bred grasshoppers and those collected in the field become infected. Jones (1936a) infected turkeys and guinea fowls with *M. lucida* after being fed cysticercoids from grasshoppers (*Melanoplus* species, *Chorthippus curtipennis,* and *Paroxya clavuliger*); chicks and quail remained negative for tapeworms after being fed cysticercoids of *M. lucida* from grasshoppers or beetles. The time required for the development of the cysticercoids in the insect host varies from 2 to 6 weeks. Approximately 3 weeks are required for the development of the adult worm to maturity in the avian host.

Pathology. Probably similar to that of *R. cesticillus.*

Davaineidae

Tapeworms of this family have a scolex with a simple rostellum which is armed
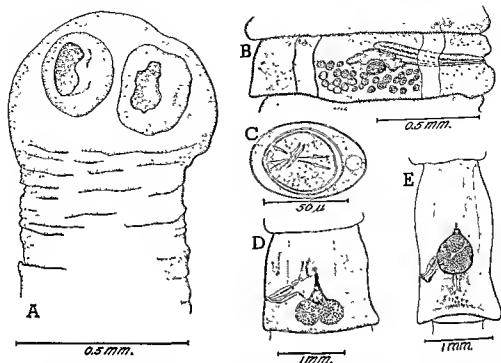
FIG. 35.3 — *Metroliasthes lucida*. (A) Scolex. Original. (B) Mature segment. (C) Egg. (D) Segment showing two-part uterus and developing paruterine organ. (E) Gravid segment. (From Ransom, 1900.)

with one or more rows of numerous hammer-shaped hooks. The suckers may be armed or unarmed. One or two sets of reproductive organs may be present in each segment. The uterus is persistent and saclike, or replaced either by numerous egg capsules or by a paruterine body transforming later into a single egg capsule.

Davainea proglottina (Davaine, 1860)

Synonym. *Taenia proglottina* Davaine, 1860.

Description. Mature worms attain a length of about 4 mm. (Fig. 35.4A). The strobila consists of from 2 to 5 segments, rarely as many as 9. Each succeeding segment gradually increases in length and breadth, the last segment often being larger than the remainder of the parasite. Suckers armed with 3 to 6 rows of small hooklets, 5 to 8μ long. Genital pores usu-

ally regularly alternate, located at extreme anterior point of segment margin. Testes 12 to 21 in number (Fig. 35.4B). One egg in each egg capsule.

In the United States this tapeworm has not been found to be as cosmopolitan in its distribution as some of the other cestodes of poultry, being found chiefly in the moister parts. It has been reported from both the eastern and western coastal states and from Tennessee and Alabama.

Life history. Cysticercoids of this tapeworm develop in approximately 3 weeks in several species of snails and slugs. Levine (1938) experimentally infected the garden slug (*Agriolimax agrestis*) with cysticercoids of *D. proglottina* and, in turn, infected chickens by feeding them garden slugs containing mature cysticercoids. When infected slugs or snails are eaten by chickens, the infective larva or cysticercoid develops to the adult worm

FIG. 35.4 – Davainea proglottina. (A) Entire worm, with eggs in last segment. (B) Mature segment. (From Meggitt, 1926.)

with 4 segments in approximately 8 days.

Pathology. This tapeworm has been considered to be one of the obviously dangerous tapeworms of poultry. It has been observed that infected birds become emaciated and dull, lose weight, the plumage becomes dry and ruffled, the movements slow, and the breathing rapid. At necropsy, the intestinal mucosa appears thickened, which may be hemorrhagic, and the intestine may contain a large quantity of mucus, which tends to be fetid. Crawley (1922) has reported this worm as killing chickens in Pennsylvania. Rietz (1930) has reported paralysis associated with the presence of this worm. However, the true relationship of leg weakness to this disease is still unknown.

Davainea meleagridis Jones, 1936

Description. Mature specimens up to 5 mm. long, composed of 17 to 22 segments. Suckers armed with 4 to 6 rows of hooklets, the longest about 5μ long; rostellum with a double row of about 100 to 130 hooks, 8 to 10μ long. Genital pores usually regularly alternate, located in extreme anterior point of segment margin. Testes 20 to 26 in number, in posterior half of segment. One egg in each capsule.

This parasite was described from the duodenum of the domestic turkey by Jones (1936b) in the vicinity of Washington, D.C., and from the wild turkey by Gardiner and Wehr (1949) in Maryland.

Life history. Unknown.

Pathology. Unknown.

Raillietina cesticillus (Molin, 1858)

Synonyms. Taenia cesticillus Molin, 1858; Raillietina cesticillus (Molin, 1858) Joyeux, 1923.

Description. Mature worms may attain a length of as much as 12 cm. Suckers unarmed; rostellum armed with two rows of hooks, about 300 to 500 in number (Fig. 35.5 A and B). Genital pores irregularly alternate, located anterior to middle of segment margin. Testes 16 to 30 in number, in posterior part of segment (Fig. 35.5C). Uterus divided into egg capsules, each capsule containing a single egg.

The most distinctive feature of this tapeworm is the unusually broad and flattened rostellum, with 2 rows of hooks near its base.

This fowl cestode is probably one of the most common species occurring in poultry. It is a rather large species, and its habitat is the duodenal and jejunal regions. Southwell (1930) reported R. cesticillus from Gallus sonnerati, the gray jungle fowl, in the Zoological Gardens of Calcutta.

Life history. Birds become infected with

FIG. 35.5 — Raillietina cesticillus. (A) Head, Original. (B) Hook from rostellum. (C) Mature segment. (From Ransom, 1905.)

R. cesticillus after being fed various infected ground beetles and dung beetles. Cysticercoids have been observed in such beetles as *Anisotarsus* spp., *Amara* spp., *Anaferonia* spp., *Harpalus* spp., *Pterostichus* spp., and other ground and dung beetles after they have been given experimental feedings of gravid segments of *R. cesticillus*, and also have been observed in natural infections in some of these beetles. Larva in beetles apparently requires from 2 to 4 weeks to develop to a stage infective for chickens. Adult worms in primary host usually require from 2 to 3 weeks to develop to maturity.

Pathology. This worm has been reported to cause degenerations and inflammations of the villi of the intestine at the point of attachment by the rostellum. Heavy infections in young birds may cause emaciation. However, Stoddard (1931) noted no serious inflammation of the intestinal walls, nor was stoppage of the intestines found to result from the presence of the worms in quail. Ackert and Reid (1937) and Ackert (1932) demonstrated experimentally that chickens 2½ to 5 months of age are more resistant to infection with this species of tapeworm than younger birds, and that a reduction in the blood sugar and hemoglobin contents of the blood resulted from such infections Harwood and Luttermoser (1938) reported that the growth rates of Rhode Island Red and White Leghorn chicks

were retarded by infections with *R. cesticillus*.

Raillietina echinobothrida (Megnin, 1881)

Synonyms. *Taenia echinobothrida* Megnin, 1881; *Raillietina echinobothrida* (Megnin, 1881) Fuhrmann, 1924.

Description. Mature specimens measure up to 25 cm. long. Suckers armed with 8 to 15 rows of hooks, 5 to 15μ long; rostellum armed with 2 rows of 200 to 240 hooks, 10 to 14μ long (Fig. 35.6 A and C). Genital pores almost unilateral, or definitely irregularly alternate, located at middle or, usually, posterior to middle of segment margin (Fig. 35.6B). Testes 20 to 30, occasionally as many as 45 in number. Uterus ultimately forming egg capsules, each capsule usually containing a single egg. Posterior segments of strobila frequently becoming constricted longitudinally through median line to form windows in the center of the segments. However, this appearance of the gravid segments is not constant in all specimens.

Raillietina echinobothrida is apparently widely distributed among poultry.

Life history. Jones and Horsfall (1935) reported that the ants *Tetramorium caespitum* and *Pheidole vinelandica* naturally harbored bladder worms or cysticercoids of *R. echinobothrida* and also those of another closely related species, *Raillietina tetragona*. The cysticercoids of the
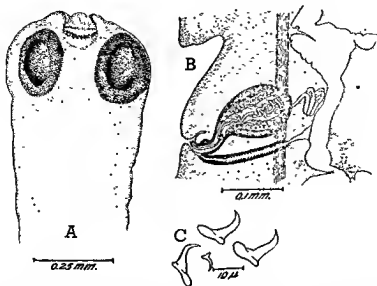
FIG. 35.6 – Raillietina echinobothrida. (A) Scolex. Original. (B) Section through region of genital pore showing cirrus pouch and part of vagina. (From Long, 1929.) (C) Hooks from suckers.

two species were fed to laboratory-reared chickens. Three weeks after feeding the cysticercoids, adults of the two species of tapeworms were recovered post mortem from the experimentally fed birds; the controls were negative. All attempts to produce experimental infections in ants were unsuccessful. Large numbers of undissected ants collected from infected poultry runs were fed to 23 chickens; 19 of the chickens later became infected. Joyeux and Baer (1937) reported finding cysticercoids of *R. echinobothrida* in naturally infected ants, *Tetramorium semileave*, in the region of Marseilles, France.

Pathology. This worm causes the formation of tubercles on the intestinal wall of infected birds (Fig. 35.7). This condition resembles tuberculosis and, therefore, must be differentiated from that disease.

Gage and Opperman (1909) reported losses of 50 per cent in affected flocks in Maryland. They noted emaciation and a mucoid diarrhea as early symptoms, and later listlessness, loss of appetite, and a tendency to huddle; some birds are weak and epileptic. Death comes suddenly, accompanied by convulsions.

Raillietina tetragona (Molin, 1858)

Synonyms. *Taenia tetragona* Molin, 1858; *Raillietina tetragona* (Molin, 1858) Joyeux, 1927.

Description. Worms measure as much as 25 cm. long. Suckers armed with 8 to 12 rows of small hooks, 3 to 8μ long; rostellum armed with about 90 to 130 hooks, 6 to 8μ long, arranged in 1 or 2 rows (Fig. 35.8A). Genital pores usually unilateral, rarely irregularly alternate, located anterior to middle of segment margin. Testes 18 to 35 in number (Fig. 35.8D). Uterus eventually breaking up into egg capsules, 6 to 12 eggs in each capsule (Fig. 35.8B).

This worm is morphologically very similar to *Raillietina echinobothrida*. It is of common occurrence but is rarely associated with the distinct tuberculosis-like lesions produced by the former species.

Life history. See life history of *R. echinobothrida*.

Pathology. Lopez-Neyra (1931) reported a single case in which he found small nodules in the intestine due to this species. In quail, Stoddard (1931) observed that this species may be the principal or only cause of death in cases of heavy in-

FIG 35.7 — Nodular disease of intestine of chicken caused by tapeworms Raillietina echinobothrida. (After Bushnell and Brandly, 1929.)

fections. Of 25 birds, the deaths of which were attributed to infection with this species, the youngest was 17 days old, and the oldest 60 days; the greatest mortality occurred between the ages of 25 and 40 days. Although many birds may recover if they survive to 2 months of age, they are almost certain to be under-sized. Quail heavily infected with specimens of this tapeworm almost invariably have their crops and gizzards filled with food. That portion of the intestine occupied by these tapeworms sometimes becomes so distended that it is reduced to nearly one-half its length, being thrown into ridges of a purplish-red color. The lining of the intestine frequently sloughs off in cases of heavy infections. In several instances Stoddard observed that bobwhites heavily infected with this species moved with difficulty, a partial paralysis being evident.

Raillietina magninumida Jones, 1930

Synonym. Raillietina (Paroniella) magninumida Jones, 1930.

Description. Mature worms about 6 to 15 cm. long. Suckers armed with about 10 rows of hooks, the largest 7 to 8μ long; rostellum armed with 2 rows of about 150 to 170 hooks, 8 to 11μ long (Fig. 35.9A). Genital pores unilateral. Testes 12 to 20 in number (Fig. 35.9B). Egg capsules containing 1 egg each.

This tapeworm is a common parasite of the guinea fowl in the United States. Hudson (1934) considered R. magninumida as a synonym of R. numida (Fuhrmann, 1912). The latter species occurs in the guinea fowl of Africa.

Life history. Guinea fowls become infected with this species by ingesting cysticercoids of this tapeworm. Cysticercoids have been found in beetles both as a result of the experimental feeding to them of gravid tapeworm segments and in natural infections. Approximately 3 weeks are required for the larva to develop to the infective stage in the beetle, and 3 weeks more are necessary for the cysticercoid to develop to the adult form in the guinea fowl.

Pathology. Adult birds seem little af-
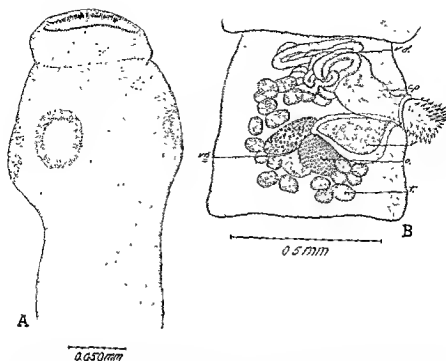
FIG. 35.9 — *Raillietina magninumida.* (A) Scolex with rostellum extended. (B) Mature segment (c.p., cirrus pouch; o., ovary; t., testes; v., vagina; v.g., vitelline gland; v.d., vas deferens). Original.
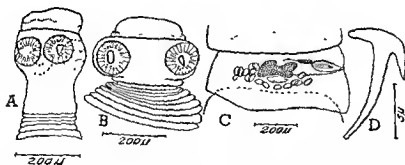
FIG. 35.10 — *Raillietina ransomi.* (A) Head fully extended. (B) Head partially contracted. (C) Mature segment. (D) Hook. (From Williams, 1931.)
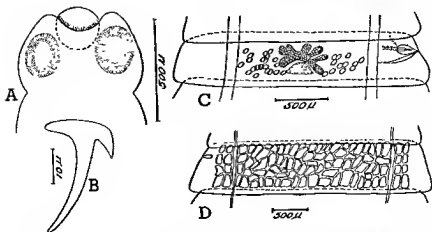
FIG. 35.11 — *Raillietina williamsi.* (A) Head with rostellum partially retracted. (B) Rostellar hooks. (C) Mature segment. (D) Gravid segment showing a single layer of egg capsules. (From Williams, 1931.)

those of the outer row being largest (Fig. 35.11A). Rostellum hemispherical, 200 to 214μ in diameter, armed with double crown of 152 to 156 hooks, larger and smaller hooks alternating (Fig. 35.11B). Genital pores unilateral, in anterior third of segment margin (Fig. 35.11C). Uterus breaking up into 75 to 100 egg capsules, each with 8 to 13 eggs (Fig. 35.11D).

This tapeworm occurs commonly in the wild turkey.

Life history. Unknown.

Pathology. Unknown.

Raillietina georgiensis Reid and Nugara, 1961

Description. Fully developed worms 150 to 380 mm. long. Suckers approximately round, with hooks 8 to 13μ long (Fig. 35.12 C); arranged in 8 to 10 circles. Rostellum armed with 220 to 268 hooks, each 17 to 23 μ long and 12 to 16μ wide (Fig. 35.12B); arranged in 2 rows. Genital pores unilateral, rarely irregularly alternate, situated in middle third of body (Fig. 35.12D). Testes 23 to 29 in number, distributed in two groups, 7 to 9 poral and 16 to 20 aporal; lying between excretory canals. Gravid proglottids longer than broad, each containing 80 to 130 egg capsules, each capsule with 8 to 10 eggs.

This tapeworm is most closely related to *R. williamsi, R. tetragona,* and *R. echinobothrida* from which it is differentiated by the size and number of rostellar hooks and in location of genital pores. It has been reported from the wild turkey in Alabama, Florida, Georgia and Tennessee, and from the domestic turkey in Georgia.

Life history. The ant, *Pheidole vinelandica* has been found naturally infected with cysticercoids (Fig. 35.12F). Domestic turkeys fed cysticercoids recovered from this ant became positive for tapeworms after about 20 days.

Pathology. Reid (1962) reported that a mild enteritis may develop in birds heavily infected with this tapeworm.

Anoplocephalidae

These worms lack both rostellum and hooks. The proglottids are usually wider than long, and each contains one or two sets of reproductive organs. The testes are numerous. The uterus may persist or be replaced by egg capsules, or the eggs may pass into one or more paruterine organs. Eggs contain "pyriform bodies."

Aporina delafondi (Railliet, 1892)

Synonyms. *Taenia delafondi* Railliet, 1892; *Bertiella delafondi* (Railliet, 1892)
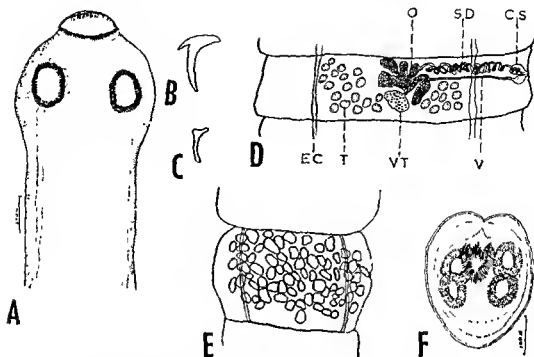
FIG. 35.12 – *Raillietina georgiensis*. (A) Scolex. (B) Rostellar hook. (C) Acetabular hook. (D) Mature segment. (E) Gravid segment. (F) Cysticercoid. (From Reid and Nugara, 1961.)

Railliet and Henry, 1909. Yamaguti (1961) transferred this species to the genus *Killigrewia* Meggitt, 1927.

Description. Mature worms 7 to 16.5 cm. long. Suckers unarmed; rostellum absent. Genital pores irregularly alternate, located in anterior third of segment margin. Testes about 100 in number. Eggs not contained in capsules.

This is a common tapeworm of pigeons in several parts of the world. In the United States it has been collected from pigeons in Iowa, Texas, Pennsylvania, and District of Columbia.

Life history. Unknown.

Pathology. Unknown.

Hymenolepididae

The hymenolepid tapeworms have a scolex with rostellum that is armed with 1 row of hooks, rarely with a double row, or unarmed. The number of testes rarely more than 4. The uterus is saclike, rarely reticulate. The eggs are enclosed in 3 envelopes.

Several species of the genus *Hymenolepis* have been transferred to other genera by the Russian helminthologists. The present writer has retained the following species in the genus *Hymenolepis*, pending a wider acceptance of the new classification.

FIG. 35.13 – *Hymenolepis carioca*. (A) Scolex. (B) Mature segment. (After Ransom, 1902.)

Hymenolepis carioca (Magalhães, 1898)

Synonyms. *Davainea carioca* Magalhães, 1898; *Weinlandia carioca* Mayhew, 1925.

This species has been placed in the genus *Echinolepis* Spassiky and Spasskaja, 1954.

Description. Mature specimens 3 to 8 cm. long, composed of many hundreds of segments; segments 3 to 5 times broader than long. Suckers and rostellum unarmed (Fig. 35.13A). Genital pores unilateral, located anterior to middle of segment margin. Testes 3 in number, usually in a more or less straight row across the segment (Fig. 35.13B).

This tapeworm is readily recognizable by its very slender and threadlike form. Complete specimens are very difficult to obtain on account of the fragility of the worm; the head is usually broken off and lost. Several thousands of these worms have been found in a single chicken.

This tapeworm is one of the most common tapeworms of the duodenum of chickens and turkeys in the United States. Stafseth (1940) reported this species of tapeworm as a parasite of quail in Michigan. Ward (1946) listed *H. carioca* as a parasite of the quail in Mississippi.

Life history. Guberlet (1919) observed that chickens became infected with this tapeworm after they had been fed stable flies caught around poultry yards. It has been demonstrated by Jones (1929) and by Cram and Jones (1929) that dung beetles act as intermediate hosts.

Horsfall (1938) successfully grew cysticercoids of this species in *Tribolium castaneum* and *T. confusum*. When flour beetles containing cysticercoids of *H. carioca* were fed to young chickens, the latter became infected with the adults of this worm. Cysticercoids develop in beetles to a stage which is infective for chickens within approximately 3 weeks. Development of the adult worm in the chicken to the time when gravid segments are passed requires from 2 to 4 weeks.

Pathology. This tapeworm sometimes occurs in large numbers in chickens and turkeys; but it has very little, if any, ef-
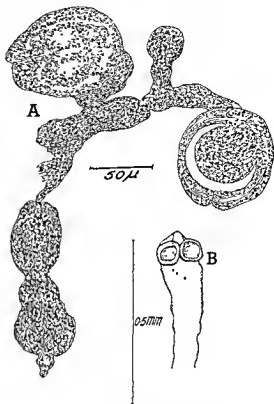
FIG. 35.14—*Hymenolepis cantaniana*. (A) Developing larvae. (B) Head. Original.

fect on the growth rate of the birds, according to Luttermoser (1940).

Hymenolepis cantaniana (Polonio, 1860)

Synonym. *Taenia cantaniana* Polonio, 1860. This species has been placed in the genus *Staphylepis* Spassky and Oshmarin, 1954.

Description. Mature specimens about 2 cm. long. Rostellum and suckers unarmed (Fig. 35.14B). Genital pores unilateral, anterior to middle of segment margin. Testes 3 in number, usually arranged in a transverse row.

This species has been reported from poultry in the United States, Puerto Rico, Europe, and Asia. It is reported from quail collected in Maryland.

Life history. The development of the cysticercoid of this species of tapeworm is rather unique. As observed by Jones and Alicata (1935), the terminal buds arise

from the many-branched individual and ultimately develop into infective larvae (Fig. 35.14A). Dung beetles serve as intermediate hosts of this tapeworm. From 2 to 3 weeks are required for the bladderworm to develop into the adult tapeworm in the avian host.

Pathology. No definite pathological conditions have been associated with this species.

Hymenolepis tenuirostris (Rudolphi, 1819)

Synonyms. *Taenia tenuirostris* Rudolphi, 1819; *Drepanidotaenia tenuirostris* (Rudolphi, 1819) Railliet, 1893. This species has been placed in the genus *Microsomacanthus* Lopez-Neyra, 1942.

Description. Mature worms 10 to 25 cm. long. Rostellum slender, with about 10 hooks, 20 to 23μ long (Fig. 35.15A). Genital pores unilateral. Testes 3 in number, in a transverse row. Eggs (Fig. 35.15B) not in capsules.

Life history. Unknown.

Pathology. Cram (1928) reported this parasite to be present in large numbers from the goose in Oregon and regarded it as responsible for heavy losses. The affected birds showed symptoms of weakness, emaciation, incoordination, and diarrhea. Gower (1939) lists this tapeworm as a parasite of the duck in North America.

FIG. 35.15 — *Hymenolepis tenuirostris.* (A) Head with rostellar hooks. (B) Egg. (From Krabbe, 1869.) (C) Cysticercoid. (From Hamann, 1889.)

Hymenolepis compressa (Linton, 1892)

Synonym. *Taenia compressa* Linton, 1892. This species has been placed in the genus *Microsomacanthus* Lopez-Neyra, 1942.

Description. Mature worms up to 4 cm. long. Suckers unarmed (Fig. 35.16A), rostellum with 10 hooks, 50 to 58μ long (Figs. 35.16B and D). Testes 3 in number, in a more or less straight row across the segment (Fig. 35.16C).

Sprehn (1932) listed this tapeworm as a parasite of ducks and geese from North America.

Life history. Unknown.

Pathology. Unknown.

Hymenolepis coronula (Dujardin, 1845)

Synonyms. *Taenia coronula* Dujardin, 1845; *Weinlandia coronula* (Dujardin, 1845) Mayhew, 1925. This species has been placed in the genus *Dicranotaenia* Railliet, 1892.

Description. Mature worms 1 to 2 cm. long. Suckers unarmed; rostellum armed with a crown of 18 to 26 hooks, 9 to 18μ long, with short handle and a strong guard which is almost as long as the blade (Fig. 35.17A and B). Testes 3 in number (Fig. 35.17C) Eggs not contained in capsules.

Life history. The eggs of this tapeworm are ingested by small crustaceans, the embryos hatching and developing to cysticercoids in the body cavity of these animals. When these infected crustaceans are swallowed by waterfowl, the cysticercoids develop to adult tapeworms in the intestines of the birds. Joyeux (1920) demonstrated that snails may carry cysticercoids of this species for a time after having eaten infected crustaceans. Birds may become infected by eating snails infected with cysticercoids.

Pathology. Pillers (1928) reported a heavy infection with this species and with *H. megalops* and *Aploparaksis furcigera* as "apparently the cause of 'going light' and deaths" in ducks in England. Kingscote (1932) reported an enzootic in a flock of ducks in Canada caused by this
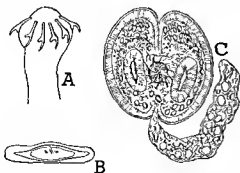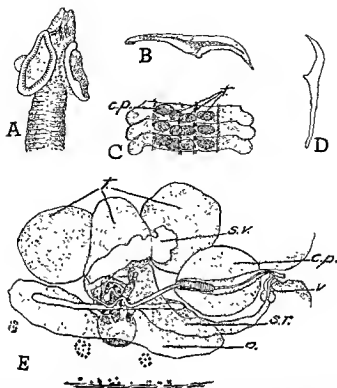
FIG. 35.16 — *Hymenolepis compressa.* (A) Head. (B) Rostellar hook. (From Linton, 1892.) (C) *Mature segments.* (D) *Rostellar hook.* (E) *Portion of transverse section through pore of mature segment* (c.p., cirrus pouch; o, ovary; s.r., seminal receptacle; s.v., seminal vesicle; t, testis; v., vagina). (From Kowalewski, 1907.)
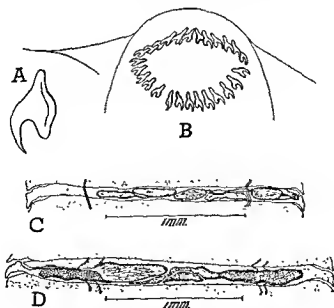
FIG. 35.17 — *Hymenolepis coronula.* (A) *Rostellar hook.* (B) *Hook crown in place.* (From Krabbe, 1869.) (C) *Mature segment with male genitalia.* (D) *Mature segment with female genitalia.* (From Meggitt, 1920.)

species, the parasites being present in large numbers. Schofield (1932) reported heavy mortality among ducklings in Canada due to *H. coronula*.

Hymenolepis lanceolata (Bloch, 1782)

Synonym. *Taenia lanceolata* Bloch, 1782. This species has been placed in the genus *Schistocephalus* Creplin, 1829.

Description. Mature worms 3 to 13 cm. long. Segments 20 to 40 times as wide as long. Suckers unarmed; rostellum with 8 hooks, 31 to 35μ long, with handle longer than blade, and guard slightly salient (Fig. 35.18C). Genital pore at anterior corner of segment margin, testes 3 in number, in a transverse row (Fig. 35.18D). Eggs not in capsules.

Quortrup and Shillinger (1941) reported *Hymenolepis* sp. (probably *H. lanceolata*) from the Canadian goose in Utah.

Life history. Ruszkowski (1932) demonstrated that larvae of this species developed to the cysticercoid stage in small crustaceans in about 6 weeks at 9°–12° C. The time required for the development of the adult worm in the primary host has not been determined.

Pathology. Emez (1929) described an epizootic, chiefly among young geese but also in some older birds. Muscular incoordination was the chief symptom. Postmortem examination showed a catarrhal inflammation of the intestinal mucosa.

Hymenolepis megalops Nitzsch, in Creplin, 1829

Synonyms. *Taenia megalops* Nitzsch, in Creplin, 1829; *Weinlandia megalops* (Nitzsch, in Creplin, 1829) Mayhew, 1925. This species has been placed in the genus *Cloacotaenia* Wolffhügel, 1938.

Description. Mature worms 3 to 6 mm. long. Head very large, 1 to 2 mm. wide (Fig. 35.19A). Suckers and rostellum unarmed. Testes 3 in number. Eggs not in capsules.

This tapeworm may be readily distinguished from other species found in poultry by its extraordinarily large head and its preference for the cloaca and bursa Fabricii. It has been found on a number of occasions in wild ducks.

Green et al. (1938) reported this tapeworm from wild ducks in Minnesota. It has been collected on a number of occasions from wild ducks in Montana by Wehr.

Life history. Unknown.

Pathology. Pillers (1923) reported a heavy infection with this worm and with *H. coronula* and *Aploparaksis furcigera* as "apparently the cause of 'going light' and of deaths in ducks in England."

Hymenolepis tritesticulata Fuhrmann, 1906

Synonym. *Weinlandia tritesticulata* (Fuhrmann, 1906). This species has been placed in the genus *Microsomacanthus* Lopez-Neyra, 1954.

Description. Mature worms 25 cm. long. Suckers unarmed; rostellum with 10 hooks, 32μ long (Fig. 35.20B). Testes 3 in number. Eggs not in capsules.

This species of tapeworm has been reported by Linton (1927) as occurring in wild ducks of North America.

Life history. Unknown.

Pathology. Unknown.

Hymenolepis introversa (Mayhew, 1925)

This species has been placed in the genus *Dicranotaenia* Railliet, 1892.

Description. Mature worms 5 to 8 cm. long. Suckers unarmed (Fig. 35.21A); rostellum armed with 20 hooks, 17 to 20μ long (Fig. 35.21B). Genital pores in anterior region of right segment margins. Testes 3 in number, irregularly lobed.

This species of tapeworm has been reported by Mayhew (1925) as occurring in the duck from Illinois.

Life history. Unknown.

Pathology. Unknown.

Fimbriaria fasciolaris (Pallas, 1781)

Synonyms. *Taenia laevis* Bloch, 1782; *Diploposthe laevis* (Bloch, 1782) Jacobi, 1896.

Description. Mature worms 10 to 50 cm.
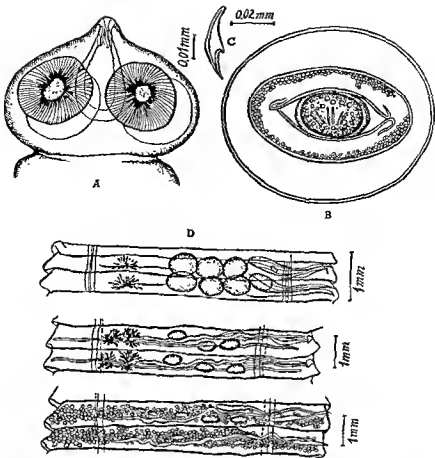
FIG. 35.18 – Hymenolepis lanceolata. (A) Head. (B) Egg. (C) Hook. (D) Proglottids in early and late stages of development. (From Potemkinot, 1938.)
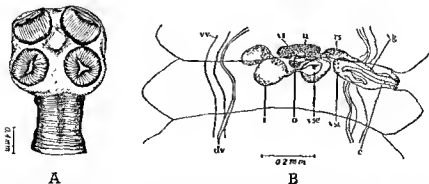
FIG. 35.19 – Hymenolepis megalops. (A) Head. (B) Mature proglottid, dorsal view (c, cirrus; dv, dorsal excretory vessel; o, ovary; ss, seminal receptacle; t, testis; u, uterus; vg, vagina; vse, vesicula seminalis externa; vsi, vesicula seminalis interna; vt, vitelline gland; vv, ventral excretory vessel). (From Yamaguti, 1940.)
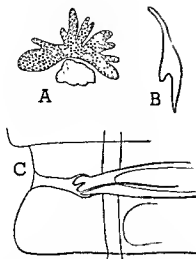
FIG 35.20 — Hymenolepis tristiculata. (A) Ovary and vitelline gland. (B) Rostellar hook. (C) Penal region showing part of cirrus pouch with internal sacculus accessorius. (From Fuhrmann, 1907.)

long by 3 to 9 mm. wide. Scolex small, provided with 10 hooks 16 to 21μ long, with long handle and very short guard and blade; suckers unarmed. Anterior part of body forms a folded expansion or "pseudoscolex." Genital pores unilateral. Testes 3 in number. Uterus continuous throughout strobila, breaking up posteriorly into tubules, each containing several eggs.

This tapeworm has been reported by Todd (1946) as occurring in chickens in Tennessee. It has also been recorded as a parasite of wild ducks on several occasions in this country.

Life history. The water flea, *Diaptomus vulgaris*, has been reported as harboring the cysticercoid of this tapeworm.

Pathology. Unknown.

SYMPTOMS

Everything else being equal, the severity of the symptoms resulting from tapeworm infections apparently depends to some extent on the number of worms present, on the diet, and on the age of the birds. Few, if any, clinical symptoms are observed in lightly infected birds. Heavily infected birds sometimes show marked retardation in growth rate.

Harwood and Luttermoser (1938) demonstrated experimentally that the growth rates of 2- to 4-week-old chicks fed an adequate diet and having infections at necropsy ranging in numbers from 15 to 155 *Raillietina cesticillus* were definitely retarded. Ackert and Case (1938) reported weight retardation and reduced sugar and hemoglobin content of the blood in 3- to 4-month-old birds each having at necropsy infections of 4 to 25 *Raillietina cesticillus*. Levine (1938) found that the difference between the mean weights of chickens experimentally infected with *Davainea proglottina* when 7 weeks of age and held under observation for 13 weeks was 12 per cent less than the controls. Alicata (1940) experimentally determined that birds receiving animal-protein supplements (fish meal and dry skim milk) had, at necropsy, an average of 14 tapeworms (*Hymenolepis exigua*), while a similar number of birds receiving plant-protein supplements (yeast, sesame meal, peanut oil, and soybean meal) had an average of 66 tapeworms. In contrast to the above observations, Luttermoser (1940) reported that the growth rates of twenty-two 4-week-old Rhode Island Red chickens experimentally fed 1,000 cysticercoids of *Hymenolepis carioca* were practically the same as those of an equal number of controls held under similar conditions.

Birds of all ages harbor tapeworms. However, Ackert and Reid (1937) have demonstrated that concomitant with an increase in age of the bird there is a corresponding increase in resistance to tapeworm infection.

A number of clinical symptoms have been inadvertently ascribed to tapeworm infections. At the present time there is not sufficient experimental evidence to show that such clinical symptoms as cyanosis, lameness, poor feathering, and failure to come into or stay in production are due solely to the presence of these parasites.

DIAGNOSIS

Diagnosis of tapeworm infection by examination of the fresh droppings for
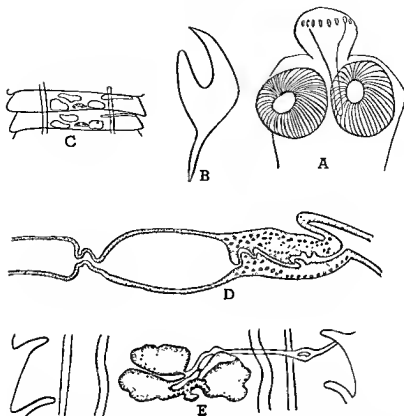
FIG. 35.21 — Hymenolepis Introversa. (A) Head. (B) Hook. (C) Proglottids. (D) Cirrus sac. (E) Reproductive organs. (From Mayhew, 1925.)

the presence of eggs or segments is unreliable. Even in cases of heavy infection, segments or eggs are sometimes absent. It has been shown by Harwood (1938) that segment production in the tapeworm *Raillietina cesticillus* occurs in cycles, segment production being marked at first by a period of intense segment elimination, alternating with periods in which no segments, or only a relatively few segments, were eliminated.

The diagnosis of poultry taeniasis is best made at necropsy. The intestine of the supposedly infected bird is slit open with an enterotome, spread out flat on the bottom of a suitable container, and examined carefully for the white ribbon-like worms. If this method of examination reveals no worms, a small amount of water may be added to the container. The water will cause the worms, if present, to float to the surface, or they may be seen swaying back and forth in the water above the opened intestine. In infections involving tapeworms of the smaller species, the individual worms are often so small that they are overlooked. Therefore, examination of the intestinal scrapings under the binocular microscope is frequently necessary to detect such small species as *Davainea proglottina* and *Amoebotaenia sphenoides*.

CONTROL OF POULTRY TAPEWORMS

Prevention. When one considers the number of tapeworms infecting poultry and their various intermediate hosts, the task of prevention of tapeworm infection in birds raised under natural conditions seems impossible. Investigations have shown that many intermediate hosts of varying habits may serve experimentally as intermediate hosts of a single species of tapeworm. To prevent birds eating the many species of invertebrates, such as insects, snails, and slugs, is an inconceivable

task. However, intermediate hosts of these parasites may be reduced in numbers by the application of certain insecticides to the poultry manure, *as shown* by Kartman *et al.* (1950). Laboratory tests conducted by these investigators showed that parathion, benzene hexachloride, chlordane, and DDT gave sufficiently satisfactory *results to warrant their use under field conditions.*

House flies may also be controlled by a spray containing 1 pint diazinon, 2 pints malathion, and 12 pounds of granulated sugar. These ingredients are mixed with enough water to make 27 gallons and should be applied lightly. One pound of technical malathion (emulsifiable concentrate) and 20 pounds of sugar give good control of both adult flies and maggots when applied to droppings, cages, and supports A commercially prepared bait containing metaldehyde will control slugs and snails. It is best to apply bait in late afternoon or evening. Ground beetles are difficult to control. These, as well as slugs and snails, tend to collect under loose boards and other protected places where it is moist. Prompt removal of these mechanical protections and elimination of moist feed wastes from poultry yards will discourage their presence in such places. Grasshoppers are controlled by spraying with the proper insecticides, poison baits, and, to some extent, by agricultural practices. The controls for stable flies, as well as house flies, are directed toward their breeding places. Moisture is necessary for the development of the fly larvae. Therefore, the prompt removal of moist feed wastes, weed piles, and other vegetation which has accumulated in piles is essential. Loose piled strawstacks, if allowed to become wet, are important breeding places of the stable fly. Hence, straw should not be allowed to become wet and decayed before removal. Ants may be controlled by the use of approved ant poisons. Malathion and Sevin may prove useful. The first step in the control of ants is to find the nests. if possible. Then an insecticide is applied to the nests and to the surfaces over which

the ants crawl. The number of earthworms may be reduced considerably by keeping the yards dry and well drained, and by avoiding the accumulation of manure.

The proper disposal of the droppings is unquestionably the most important single preventive measure for the control of tapeworm infection. The droppings of infected birds are the source from which the intermediate hosts become infected. Therefore, care in removing the droppings frequently and disposing of them in such manner as to prevent the intermediate hosts from picking up the tapeworm eggs or gravid segments passed in the droppings is of primary importance. The body wastes from farm flocks can usually be disposed of by hauling them to the field and spreading thinly over the land. The action of the sun and wind will quickly dry out the droppings and destroy all parasitic material that may be present in them, since prolonged desiccation is fatal to the infective stages of the parasites. This practice of disposing of poultry droppings not only serves to destroy parasitic material but also adds tremendously to the value of the land for growing crops. Poultry manure, when handled in this way, is said to be an excellent fertilizer for garden and field crops.

The body wastes from backyard flocks usually must necessarily be handled in some other way, as accumulations from such flocks usually exceed the demands of the owner. In communities where large numbers of poultry raisers are within a short distance of each other, the droppings from their birds are sometimes hauled to one or more centrally located storage sheds and retailed to the public at a reasonable price. In order that the fertilizing value of poultry manure may not be lost, it must be stored in a suitable screened-in shed which has been provided with a cement floor and with a roof to keep out the rain and snow. The screens exclude the flying and crawling insects which may serve as intermediate hosts of poultry parasites. This practice naturally

raises the question as to how safe this manure is if used on land where other poultry are likely to run. Limited experimentation seems to indicate that much destruction of the tapeworm eggs may result from the self-sterilization process which takes place in stored manure.

TREATMENT

Medication has served to reduce appreciably parasitism in many groups of livestock, but its applicability in the control of poultry parasites in general is limited.

A drug, in order to be a satisfactory poultry remedial agent, must be inexpensive, highly effective, nontoxic, and easy to administer. It is highly essential that a drug designed for the purpose of removing parasites from poultry possess the above qualifications, since the unit value of the domestic fowl is usually quite low.

A large number of drugs have been recommended for the removal of tapeworms from poultry. Guthrie and Harwood (1941) reported that mixtures of 0.3 to 1.0 gm. of stannous (tin) tartrate and 0.07 to 0.2 gm. of synthetic pelleterine hydrochloride removed 86.8 per cent of *Raillietina cesticillus* and 95.0 per cent of *Hymenolepis carioca* from experimentally infected chickens. However, when used by themselves, the tin compounds possessed only slight value for the removal of *R. cesticillus*. By adding small amounts of synthetic pelleterine hydrochloride to any of the various tin compounds, a synergistic action was obtained, thus increasing the effectiveness of the mixture. The above authors (1944) found that a freshly prepared mixture of tin oleate and triethanolamine effectively removed a large percentage of the *R. cesticillus* from experimentally infected birds in some tests and only a small percentage of these worms in others. Kerr (1948) demonstrated that hexachlorophene (2,2'-dihydroxy-3,3', 5, 5', 6, 6'-hexachloro-diphenylmethane) at doses of 25–50 mg. per kg. body weight possesses a high efficacy in removing *Raillietina cesticillus* from chickens. However, when used at the therapeutic level

this drug seriously affects egg production.

Kerr (1952) presented data to show that butynorate was an effective and safe drug for the removal of *Raillietina cesticillus* from chickens. When administered as a single dose by capsule, a dose of 75 to 150 mg. per kilogram of body weight gave efficacies ranging from 86 to 100 per cent. Edgar (1956) reported the above compound to be highly effective in removing six species of tapeworms, *R. cesticillus, R. tetragona, Hymenolepis carioca, Choanotaenia infundibulum, Davainea proglottina,* and *Amoebotaenia sphenoides,* from field-infected chickens when administered in the feed at the rate of 500 mg. per kilogram of feed for 2 to 6 days, or by capsule at the rate of 125 mg. per bird, or in combination with nicotine and phenothiazine. A temporary drop in egg production, which persisted from the third through the tenth day after treatment, resulted from the use of the combination.

Nugara and Reid (1962) tested Trithiadol in the feed at the rate of 3 pounds per ton for 5 days, dibutyltin dilaurate (butynorate) in the feed at a 0.07 per cent level for 5 days, and dibutyltin oxide via capsule at 65 mg. and 125 mg. doses per bird against the turkey tapeworm, *Raillietina georgiensis.* They reported the latter to be the most efficacious, removing 100 per cent of the worms at the lower level and 90 per cent at the higher level.

Reid (1940) found that starvation of birds infected with the cestode *Raillietina cesticillus* for 20 to 48 hours, including the overnight feeding intervals of the chicken, resulted in the loss of the strobilae (minus the head) of the worms. The loss of the tapeworm strobilae was apparently directly due to the partial starvation of the parasite, as it was determined that the glycogen store in worms from chickens starved 20 hours was lowered to less than one-twelfth of that found in tapeworms taken from nonstarved birds. However, he (Reid, 1942) demonstrated that the tapeworm head was not affected by the long period of starvation. When normal

feeding habits of the fowl were restored, new strobilae or segments were regenerated by the unaffected heads, and gravid segments appeared later in the feces of the birds. Therefore, it is obvious that the practice of starving tapeworm-infected birds has the effect of breaking off the

strobilae and leaving the heads attached to the mucosa. Because of its harmful effects on the health and growth of the birds and the rapid regeneration of new segments following the starvation period, such a procedure is not practical.

REFERENCES

Abdou, A. H.: 1958. Studies on the development of *Davainea proglottina* in the intermediate host. Jour. Parasit. 44:484.

Ackert, J. E.: 1919. On the life cycle of the fowl cestode, *Davainea cesticillus* (Molin). Jour. Parasit. 5:41.

———. 1932. Fowl resistance to parasitism affected by vitamins A and B. Arch. Zool. Ital. Torino 16:1369.

———, and Case, A. A.: 1938. Effects of the tapeworm *Raillietina cesticillus* (Molin) on growing chickens. Jour. Parasit. 24:44.

———, and Reid, W. M.: 1936. The cysticercoid of the fowl tapeworm, *Raillietina cesticillus*. Trans. Amer. Micros. Soc. 55:97.

———, and Reid, W. M. 1937. Age resistance of chickens to the cestode *Raillietina cesticillus* (Molin). Jour. Parasit. 23:558.

Adams, F. M., and Geser, S. W.: 1933. Helminth parasites of the chicken, *Gallus domesticus*, in Dallas County, Texas. Am. Midl. Nat. 14:251.

Alicata, J. E.: 1936. The amphipod, *Orchestia platensis*, an intermediate host for *Hymenolepis exigua*, a tapeworm of chickens in Hawaii. Jour. Parasit. 22:515.

———. 1940. Poultry parasites. Annual Report, Hawaii Agr. Exper. Sta. (1959).

———, and Chang, E.: 1939. The life history of *Hymenolepis exigua*, cestode of poultry in Hawaii. Jour. Parasit. 25:121.

———, and Jones, M. F.: 1933. The dung beetle, *Ataenius cognatus*, as the intermediate host of *Hymenolepis cantaniana*. Jour. Parasit. 19:244.

Case, A. A., and Ackert, J. E.: 1939. Intermediate hosts of chicken tapeworms found in Kansas. Trans. Kans. Acad. Sci. 42:437.

———, and Ackert, J. E.: 1940. New intermediate hosts of fowl tapeworms. Trans. Kans. Acad. Sci. 43:393.

Chandler, A. C.: 1935. Observations on the life cycle of *Davainea proglottina* in the United States. Trans. Amer. Micros. Soc. 42:141.

Cram, E. B.: 1928. The present status of our knowledge of poultry parasitism. No. Am. Vet. 9:43.

———, and Jones, M. F.: 1929. Observations on the life histories of *Raillietina cesticillus* and of *Hymenolepis carioca*, tapeworms of poultry and game birds. No. Am. Vet. 10:49.

Crawley, H.: 1922. *Davainea proglottina*, a pathogenic cestode, in American poultry. Jour. Am. Vet. Med. Assn 61:305.

Cuvillier, E., and Jones, M. F.: 1933. Two new intermediate hosts for the poultry cestode, *Hymenolepis carioca*. Jour. Parasit. 19:245.

Edgar, S. A.: 1956. The removal of chicken tapeworms by di-n-butyl tin dilaurate. Poultry Sci. 35:64.

———, and Teer, P. A.: 1957. The efficacy of several compounds in causing the elimination of tapeworms from laboratory-infected chickens. Poultry Sci. 36:329.

Emez, S.: 1929. (Cerebellar ataxia in geese as a result of infestation with *Hymenolepis lanceolata*.) (Russian text.) Vestnik Sovrem Vet. Moskva (94). Vol. 5 (21). Nov., p. 531.

Engle, K., and Steinsky, E.: 1959. Zur biologie und bekampfung der haulgsten hühnerbandwurmer Arch. Geflugelk. 23:247.

———, and Steinsky, E.: 1959. Die Zwischenwirte der hühnerbandwurmer *Raillietina cesticillus*, *Choantaenia infundibulum* and *Hymenolepis carioca* Z. Parasitenk. 19:278.

———, Steinsky, E., and Ergun, H.: 1958. Die Zwischenwirte von *Davainea proglottina* (Cestoidea) Z. Parasitenk. 18:320.

Ferry, G. B.: 1934. Studies on cestoda of poultry found in and around Douglas County, Kansas. Am. Midl. Nat. 15:586.

Gage, G. E., and Opperman, C. L.: 1909. Nodular taeniasis, or tapeworm disease, of fowls. Md. Agr. Exper. Sta. Bul. 139 73.

Gardiner, J. L., and Wehr, E. E.: 1949. Some parasites of the wild turkey (*Meleagridis gallopavo silvestris*) in Maryland. Proc. Helminth. Soc. Wash. 16:16.

Gower, W. C.: 1939. Host-parasite catalogue of the helminths of ducks. Am. Midl. Nat. 22(3):580.

Grassi, B., and Rovelli, G.: 1889. Embryologische Forschungen an Cestoden. Zentralbl. f. Bakt. u. Parasitenk. 5.370 and 401.

Green, R. G., *et al.*: 1938. The occurrence of botulism in waterfowl in western Minnesota. Minn. Wildlife Dis. Invest. 3:123.

Guberlet, J. E.: 1919. On the life history of the chicken cestode, *Hymenolepis carioca* (Magalhães). Jour. Parasit. 6:35.

Guthrie, J. E., and Harwood, P. D.: 1941. Use of tin preparations for the treatment of chickens experimentally infected with tapeworms. Am. Jour. Vet. Res. 2 108.

——, and Harwood, P. D.: 1944. Limited tests of mixtures of tin oleate with ammonium compounds for the removal of experimental tapeworm infections of chickens. Proc. Helminth. Soc. Wash. 11:45.

Hanson, A. J.: 1930. The slug as the intermediate host of the microscopic tapeworm of chickens. Ann. Rept. Western Wash. Exper. Sta.

Harkema, R.: 1943. The cestodes of North Carolina poultry with remarks on the life history of *Raillietina tetragona*. Jour. Elisha Mitchell Sci. Soc. 59 127

Harwood, P. D.: 1938. Reproductive cycles of *Raillietina cesticillus* of the fowl. Livro Jub. Lauro Travassos, p. 213. Rio de Janeiro, Institute Oswaldo Cruz.

——, and Guthrie, J. E.: 1940. Tests with miscellaneous substances for removal of tapeworms from chickens. Jour. Am. Vet. Med. Assoc. 97:248.

——, and Luttermoser, G. W.: 1938. The influence of infections with the tapeworm, *Raillietina cesticillus*, on the growth of chickens. Proc. Helminth. Soc. Wash. 5:60.

Horsfall, M. W.: 1938. Meal beetles as intermediate hosts of poultry tapeworms. Poultry Sci. 17:8

——, and Jones, M. F.: 1937. The life history of *Choanotaenia infundibulum*, a cestode parasitic in chickens. Jour. Parasit. 23:435.

Hudson, J. R.: 1934. Notes on some avian cestodes. Ann. and Mag. of Nat. Hist., Series 10 (80), 14:514.

Jones, M. F.: 1929. *Hister (Carcinops) 14-striatus* an intermediate host for *Hymenolepis carioca*. Jour. Parasit. 15:224.

——: 1930a. A new tapeworm from the guinea fowl, with cysticercoids in a ground beetle. Jour. Parasit. 16:158.

——: 1930b. Life history of *Metroliasthes lucida*, a tapeworm of the turkey. Jour. Parasit. 17:33.

——: 1932. Additional notes on intermediate hosts of poultry tapeworms. Jour. Parasit. 18:307.

——: 1936a. *Metroliasthes lucida*, a cestode of galliform birds, in arthropod and avian hosts. Proc. Helminth. Soc. Wash. 3:26.

——: 1936b. A new species of cestode, *Davainea meleagridis* (Davaineidae), from the turkey, with a key to species of Davainea from galliform birds. Proc. Helminth. Soc. Wash. 3:49.

——, and Alicata, J. E.: 1935. Development and morphology of the cestode, *Hymenolepis cantaniana* in coleopteran and avian hosts. Jour. Wash. Acad. Sci. 25:339.

——, and Horsfall, M. W.: 1935. Ants as intermediate hosts for two species of Raillietina parasitic in chickens. Jour. Parasit. 21:442.

Joyeux, C.: 1920. Cycle évolutif de quelques cestodes. Recherches expérimentales. Bul. de l'Inst. Pasteur 18:346.

——, and Baer, J. G.: 1937. Recherches sur l'évolution des cestodes de gallinacés. Compt Rend. Acad. Sci. 205:751.

Kartman, L., Tanada, Y., Holdaway, F. G., and Alicata, J. E.: 1950. Laboratory tests to determine the efficacy of certain insecticides in the control of arthropods inhabiting poultry manure. Poultry Sci. 29:336.

Kerr, K. B.: 1948. Hexachlorophene as an agent for the removal of *Raillietina cesticillus*. Poultry Sci. 27:781.

——: 1952. Butynorate, an effective and safe substance for the removal of *R. cesticillus* from chickens Poultry Sci. 32.328.

Kingscote, A. A.: 1932. Department of Parasitology. Rep. Ontario Vet. Coll. (1931) p. 60.

Levine, P. P.: 1938. The effect of infection with *Davainea proglottina* on the weights of growing chickens. Jour. Parasit. 24:550.

Linton, E.: 1927. Notes on cestode parasites of birds. Proc. U.S. Nat. Mus. (2656) 70, Art. 7, 73 pp. pls., 1–15, figs. 1–221.

Lopez-Neyra, C. R.: 1931. Revision del genero Davainea. Mem. Acad. Cien. Exact., Fes. y Nat. Madrid, s. Cien. Nat. 1:1.

Luttermoser, G. W.: 1940. The effect on the growth-rate of young chickens of infections of the tapeworm, *Hymenolepis carioca*. Proc. Helminth. Soc. Wash. 7:74.

Mayhew, R. L.: 1925. Studies on the avian species of the cestode family Hymenolepididae. Ill. Biol. Monogr. 10(1), Jan., pp 1–125, figs. 1–7, pls. 1–9, figs. 1–11.

Meggitt, F. J.: 1914. On the anatomy of a fowl tapeworm *Amoebotaenia sphenoides*. Parasitology 7.262.

——: 1916. A contribution to the knowledge of the tapeworms of fowls and of sparrows. Parasitology 8:390.

——: 1926. The tapeworms of the domestic fowl. Jour. Burma Res. Soc., Rangoon 15:222.

Moenig, H. O.: 1927. The anatomy and life history of the fowl tapeworm *Amoebotaenia sphenoides*. Union of So. Africa, Dept. of Agr.. 11th and 12th Rep. Dir. Vet. Educ. and Res.. Pt. 1:199.

Nugara, D., and Reid, W. M.: 1962 Some drug treatments for the turkey tapeworm, *Raillietina georgiensis*. Poultry Sci. 41:674.

Pillers, A. W. N.: 1923. Notes on parasites during 1922 Vet. Record 5:459.

Quortrup, E. R., and Shillinger, J. E.: 1941. 3,000 wild bird autopsies on western lake areas. Jour. Am. Vet. Med. Assn. 99:382.

Ransom, B. H.: 1900. A new avian cestode—*Metroliasthes lucida*. Trans. Amer. Micros. Soc. 21:213.

———: 1902. On *Hymenolepis carioca* (Magalhaes) and *H. megalops* (Nitzsch) with remarks on the classification of the group. Trans. Amer. Micros. Soc. 23:151.

———: 1905. The tapeworms of American chickens and turkeys. 21st Ann. Rep. Bur. Anim. Ind., U.S.D.A. (1904):268.

Reid, W. M.: 1940 Some effects of short starvation periods upon the fowl cestode *Raillietina cesticillus* (Molin). Jour. Parasit. 26 (suppl.):16.

———: 1942. The removal of the fowl tapeworm *Raillietina cesticillus* by short periods of starvation. Poultry Sci. 21:220.

———: 1959. Egg characteristics as aids in species identification and control of chicken tapeworms. Avian Dis. 3:188

———: 1962. Chicken and Turkey Tapeworms. Handbook to aid in identification and control of tapeworms found in the United States of America. Univ. of Georgia, College of Agriculture, June.

———, Ackert, J. E., and Case, A. A.: 1938 Studies on the life history and biology of the fowl tapeworm *Raillietina cesticillus* (Molin) Trans Amer. Micros. Soc. 57,65.

———, and Nugara, D.: 1961. Description and life cycle of *Raillietina georgiensis* n. sp., a tapeworm from wild and domestic turkeys. Jour. Parasit 47,885.

Rietz, J. H.: 1930. Animal parasites of chickens in Ohio and West Virginia. Jour. Am. Vet. Med. Assn. 77:151

Ruszkowski, J S.: 1932. Cycle d'évolution du cestode *Drepanidotaenia lanceolata*, Acad. Polon. Sc. et Lett., Compt Rend. Mens. Cl Sc Math. et Nat., Cracovie, (1), Jan., p. 4.

Sawada, I.: 1952. Ants as intermediate hosts for chicken tapeworm, *Raillietina tetragona*, Nara Gakugei Univ. Bul 1:225. English summary.

———: 1952 On the life history of the chicken cestode, *Raillietina cesticillus*. Nara Gakugei Univ. Bul 1 255 English summary.

———: 1953 Observation on the seasonal variation in infestation rate of cysticercoids of *Raillietina tetragona* and *Raillietina echinobothrida* in the ant, *Tetramorium caespitum* racoti. Zool Mag 62 292 English summary.

———: 1954 Morphological studies on the chicken tapeworm, *Raillietina (Raillietina) echinobothrida* Zool Mag. 63 200 English summary

Schofield, F. W. 1932. Heavy mortality among ducklings due to *Hymenolepis coronula*. Rep Ontario Vet Coll (1931),49

Schwartz, B 1925 The chicken as a host for *Metroliasthes lucida*. Jour. Parasit. 12:112.

Southwell, T: 1921 Cestodes from Indian poultry. Ann. Trop Med. and Parasit. 15:161.

———: 1930 Cestoda The Fauna of British India, including Ceylon and Burma. 2,9.

Sprehn, C. E. W.: 1932. Lehrbuch der Helminthologie. Eine Naturgeschichte der in deutschen Säugetieren und Vögeln schmarotzenden Würmer, unter besonderer Berücksichtigung der Helminthen des Menschen, der Haustiere und wichtigsten Nutztiere. 998 pp., figs. 1–574. Berlin

Stafseth, H. J.: 1940. Tapeworm infestation in poultry. Poultry Practice. A collection of discussions on poultry diseases and related subjects Reprinted from Vet. Med. 34:765.

Stoddard, H. L.: 1931. The Bobwhite Quail, Its Habits, Preservation, and Increase. Charles Scribner's Sons, New York

Suppérer, R.: 1954 Versuche ueber die Entwicklung des Geflügel-Bandwurmes *Hymenolepis cantaniana* Polonio, 1860 Wien Tierärztl. Monatschr. 41:199.

Todd, A. C.: 1946. The nature of helminth infestations in chickens in East Tennessee. Poultry Sci. 25,424.

Ward, J. W.: 1946. A preliminary study of the occurrence of internal parasites in chickens in Mississippi. Proc. Helminth. Soc Wash. 13:12

Wardle, R. A., and McLeod, J A 1952 The Zoology of Tapeworms. Univ. of Minnesota Press, Minneapolis, 780 pp. 173, 174.

Wehr, E. E., and Coburn, D. R 1943 Some economically important parasites of the wild turkey and Hungarian partridge of Pennsylvania. Pa Game News 13:14 and 31.

Wetzel, R.: 1932. Zur Kenntnis des wenigghedrigen Huhnerbandwurmes *Davainea proglottina*. Arch. Wissensch. u. Prakt. Tierh. 65:595.

———: 1933. Zur Kenntnis des Entwicklungskreises der Huhnerbandwurmes *Raillietina cesticillus*. Deutsche Tierärztl. Wochenschr 41:465.

Williams. O. L.: 1931. Cestodes from the eastern wild turkey. Jour. Parasit. 18:14.

Yamaguti, O.: 1961. The cestodes of vertebrates In S Yamaguti, Systema Helminthum, Inter-science Publ, New York

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36

Trematodes of Poultry

The trematodes, or flukes, are parasitic flatworms that as adults are devoid of cilia or other locomotor appendages, but are provided with adhesive organs in the form of suckers or other specialized structures.

The class TREMATODA is usually divided into two subclasses, namely, the MONOGENEA and the DIGENEA. Some systematists recognize a third subclass, ASPIDOGASTREA, which comprises a peculiar group of flukes usually parasitic in bivalve mollusks. The MONOGENEA are parasites of cold-blooded animals, as a rule, and usually live on the exterior of the body; they are peculiar forms having elaborate adhesive organs and direct life histories. The DIGENEA are almost exclusively endoparasitic and, for the most part, are provided with adhesive organs in the form of suckers; the life histories are complex, involving alternation of generations and of hosts.

The digenetic trematodes are customarily divided into two orders, GASTEROSTOMATA and PROSOSTOMATA. The GASTEROSTOMATA is a relatively small group consisting of several genera characterized by having a single, saclike digestive tract communicating with the exterior through a mouth located near the middle of the ventral surface of the body. The gasterostomes are parasites of fishes. The PROSOSTOMATA is the order to which all of the poultry flukes belong. Members of this group are characterized by having the mouth located at or near the anterior end of the body. The mouth is usually surrounded by a sucker; a second sucker is usually present on the ventral surface near the middle or, more rarely, at the posterior end of the body.

General morphology. In general the body of the adult fluke is leaflike, occasionally cylindrical, and frequently covered with scalelike spines. Except for the blood flukes (Schistosomatidae), all trematodes of poultry are hermaphroditic, that is,

both the male and female organ systems are present in a single individual. The male reproductive system usually consists of two testes, vasa efferentia, a vas deferens which enlarges to form a seminal vesicle, and a copulatory organ or cirrus surrounded by a saclike structure known as the cirrus pouch. The female system consists of an ovary, vitelline or yolk glands, an ootype or chamber in which the ovum and yolk cells are surrounded by shell material, and a long slender uterus, the terminal portion of which is modified to form a vagina or metraterm. Both the male and female ducts usually open into a cavity or genital sinus which communicates with the exterior through the genital pore. In most flukes the genital pore is situated ventrally in the anterior part of the body. The digestive system is simple, and consists of a mouth, a short tube or prepharynx, a muscular bulb or pharynx, and a slender esophagus of varying length which branches to form the intestine; the intestinal branches are usually simple blind sacs or ceca, but in some forms the two branches are fused posteriorly (Cyclo-coelidae) or united and terminating in a common cecum (Schistosomatidae). The nervous system consists of ganglia located in the pharyngeal region and of anteriorly and posteriorly directed nerves. The excretory system consists of an excretory pore that is located at the posterior end of the body, a bladder, two principal collecting ducts, and collecting tubules which ramify and terminate in flame cells.

Development. The developmental cycles of the trematodes of poultry are very complex. The eggs that are passed by the mature flukes customarily reach the exterior in the feces. On reaching water the eggs undergo embryonation and, in the course of time, hatch. The embryo or *miracidium* thus liberated swims about in search of a snail intermediate host. In some instances, as in the Cyclocoelidae and Schistosomatidae, the egg contains a fully formed miracidium at the time it is laid, and hatching takes place soon after

it reaches water. In other instances, as in the Opisthorchiidae and Brachylaemidae, the egg contains, at the time of deposition, a fully formed miracidium which is not liberated until the egg is ingested by the snail host. On reaching a suitable location in the snail's tissues, the miracidium is transformed into a *sporocyst*. When fully developed, the sporocyst may give rise to a larva provided with a mouth and gut, which is known as a *redia*, and to *cercariae* (Echinostomatidae and Paramphistomidae), or it may give rise to *daughter sporocysts* and cercariae (Schistosomatidae and Strigeidae). The cercaria consists of a body, which becomes the mature fluke, and a tail which enables it to swim about. In some instances the cercaria may become encysted in the water or on various objects (Paramphistomidae and Notocotylidae) or penetrate into secondary intermediate hosts, such as snails, tadpoles, and fishes (Echinostomatidae, Opisthorchiidae, and Strigeidae), and become encysted. The encysted cercaria is known as a *metacercaria*. When the young encysted fluke, or metacercaria, is eaten by the final or definitive host, the cyst wall is digested, and the young fluke is liberated and grows to maturity. In the Schistosomatidae the metacercarial stage is omitted; the cercaria penetrates the skin of the definitive host and on reaching the circulatory system develops into the adult fluke.

Importance of flukes as parasites of poultry. In comparison with the nematodes or roundworms of poultry, the flukes are of much less importance. In spite of the fact that a large number of trematodes are known from poultry — about 50 species from the chicken, 12 from the turkey, 8 from the guinea fowl, 2 from the peafowl, 28 from the pigeon, 75 from the duck, and 24 from the goose — only a few have been reported as causing serious injury. Many of the flukes of poultry have disease-producing potentialities, and serious losses may result if infections are sufficiently large. In the case of the trematodes, as with

other parasites, the amount of damage produced depends largely on the number of individuals harbored and to a lesser degree on the organs affected. In this chapter consideration is given mainly to those trematodes occurring in the United States which are actually or potentially capable of causing serious loss.

The species of flukes parasitizing poultry belong to 18 families, the more important of which may be distinguished by the following key:

1. Sexes separate; parasites of the circulatory system . . . Schistosomatidae
   Hermaphroditic; not parasites of the circulatory system 2

2. Body fleshy, rounded or hemispherical; in cysts of skin
   . . . . Troglotrematidae
   Body elongated, usually flattened; not in cysts 3

3. Intestinal branches united posteriorly; parasites of respiratory system . . . . Cyclocoelidae
   Intestinal branches not united posteriorly 4

4. With oral sucker only 5
   With both oral and ventral suckers 6

5. Pharynx absent; uterus pretesticular; parasites of intestine and ceca . . . . Notocotylidae
   Pharynx present; uterus largely post-testicular; parasites of kidney . . . . Eucotylidae

6. Acetabulum or ventral sucker located at posterior end of body . . . . Paramphistomidae
   Acetabulum or ventral sucker in middle, or anterior to middle, of body 7

7. Uterus passing between testes, reaching posterior end of body . . . . Plagiorchiidae
   Uterus pretesticular 8

8. Cirrus pouch absent; parasites of bile ducts . . . . Opisthorchiidae
   Cirrus pouch present; not parasites of bile ducts 9

9. Body divided by constriction into a cup-shaped anterior portion and a cylindrical posterior portion . . . . . . Strigeidae
   Body not divided as above 10

10. Gonads in posterior fourth of body; ovary between testes . . . . Brachylaemidae
    Gonads in middle, or posterior to middle of body; ovary in front of testes 11

11. Oral sucker surrounded by an adoral disc armed with relatively large spines . . . . Echinostomatidae
    Oral sucker not surrounded by an adoral disc 12

12. Vitellaria tubular; eggs containing eye-spotted miracidia when deposited; parasites of the conjunctival sac . . . . Philophthalmidae
    Vitellaria follicular; eggs not containing eye-spotted miracidia when deposited; parasites of digestive tract . . . . . Psilostomidae

TREMATODES OF THE SKIN

The skin fluke belongs to the Troglotrematidae. Members of this family have more or less plump, spiny bodies and frequently occur in cysts, usually in pairs.

Collyriclum faba (Bremser, 1831)

Synonym. *Collyriclum colei* Ward, 1917.

Description. Body hemispherical, 4.2 to 8.6 mm. long by 4.5 to 5.5 mm wide (Fig. 36 1). Oral sucker subterminal; acetabulum absent. Testes variable in shape, ovary T shaped, with each of the branches divided into several lobes Vitellaria in anterior part of body, somewhat asymmetrical, consisting of 6 to 9 groups of follicles on each side. Uterus greatly coiled, in posterior part of body. Eggs 19 to 21μ long by 9 to 11μ wide.

This parasite occurs encysted in the skin of chickens and turkeys and of a number of passerine birds. The cysts are 4 to 6 mm. in diameter, and each contains two flukes, one usually smaller than the other An opening is present at the summit of the cyst through which the eggs of the flukes escape. In the United States this fluke has been reported from poultry in Minnesota where it was found in young chickens and turkeys by Riley and Kernkamp (1924). This parasite has also been reported by Marotel (1926) as parasitizing turkey poults in southeastern France.

Life history. The life history of this fluke is unknown. Like other members of the Troglotrematidae, this fluke undoubtedly

FIG. 36.1 — Collyriclum faba. Ventral view. (From Kossack, 1911.)

requires a snail primary intermediate host and a secondary intermediate host, which is probably an arthropod. The theory of Jegen (1917) that infection is direct, since he reported that the eggs contained two embryos which were not miracidia but young flukes, is disproved by Tyzzer (1918), Riley and Kernkamp (1924), and Riley (1931) who observed miracidia escaping from the eggs, as in the case of other flukes. Riley is of the opinion that dragonfly larvae may serve as secondary intermediate hosts because the outbreaks of infection among chickens and turkeys which he was able to observe occurred in birds having access to wet or marshy places at a time in early summer when the dragonfly nymphs were emerging. Riley also "recovered from these nymphs metacercariae which suggest closely the characteristics of the adult Collyriclum."

Pathology. The encysted flukes are found mainly around the vent, but may occur elsewhere on the body of the affected bird (Fig. 36 2). In the cases studied in the United States there were no striking symptoms, but there is little doubt that extremely heavy infections in young birds would prove fatal. In poultry of marketable age the presence of Collyriclum cysts would greatly decrease the value of the birds.

TREMATODES OF THE EYE

The eye flukes belong to the family Philophthalmidae. They are relatively small trematodes with well-developed suckers and without spines; the gonads are in the posterior end of the body, the ovary being in front of the testes; the yolk glands or vitellaria are tubular.

Philophthalmus gralli Mathis and Léger, 1910

Description. Body lanceolate, 3 to 6 mm. long by 0.9 to 1.7 mm. wide, yellowish and transparent. Oral sucker 0.285 mm. wide; acetabulum about 0.588 mm. in diameter, about one-fourth of body length from anterior end. Genital aperture about midway between oral sucker and
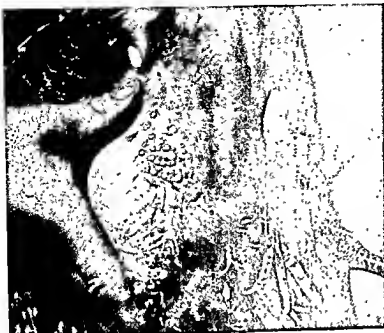
FIG. 36.2 — View of abdomen of turkey showing cysts of Collyiclum faba. (Kernkamp, Univ. of Minn.)

acetabulum. Cirrus pouch slender, its base slightly distal to acetabulum. Testes oval, tandem, in posterior fourth of body. Ovary median, pretesticular. Uterus with numerous transverse loops, filling greater part of body from level of anterior margin of testicular zone to base of cirrus pouch; metraterm slender, as long as, and paralleling, cirrus pouch. Vitellaria tubular, more or less obscured by uterus. Eggs in uterus 85 to 120μ long by 39 to 55μ wide, containing fully developed miracidia.

Life history. The life cycle (Fig. 36.3) has been ascertained by Alicata and Noda (1960) and Alicata (1962) in Hawaii, and by West (1961) in Indiana. The egg when laid contains a fully developed miracidium in which is found a mother redia. Hatching takes place almost immediately on reaching water. Upon coming in contact with a suitable snail (*Tarebia granifera mauiensis* and *Melanoides newcombi* in Hawaii, and *Goniobasis* spp. and *Pleurocerca acuta* in Indiana) the miracidium penetrates and the mother redia escapes into the heart. According to Alicata, two subsequent generations of redia develop in the heart and digestive gland, the final generation giving rise to cercariae. The

time required for development from mother redia to cercaria is 3 months or longer. Upon emergence from the snail host, the cercariae soon encyst on any solid object, including the shells of snails and exoskeletons of crayfish. When eaten by
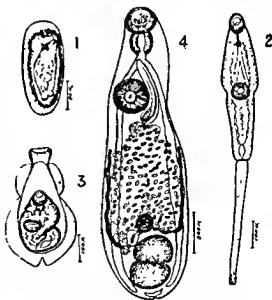
FIG. 36.3 — Philophthalmus gralli. (1) Egg. (2) Cercaria. (3) Encysted metacercaria. (4) Adult. (From Alicata and Noda, 1960.)

a bird host, the encysted cercaria (meta-cercaria) excysts in the mouth and crop, the young flukes migrate to the conjunctival sac through the naso-lacrimal duct; from 1 to 5 days are required for the excysted metacercariae to reach the eye where they become mature in about a month.

Pathology. The attachment of the worms by their suckers to the conjunctiva causes congestion and erosion of the membrane. The conjunctival fluid contains blood, fluke eggs, and active miracidia.

Philophthalmus gralli has been reported in natural infections of the chicken, peafowl, turkey, duck, and goose in Indo-China and Formosa, and in experimental infections of chicks in the United States (Hawaii and Indiana). Several other species have been reported from domestic poultry as follows: *P anatinus* Sugimoto from the duck in China, *P mnraschkinzcwi* Tretiakowa from the duck in Russia, and *P. problematicus* Tubangui from the chicken and *P. rizalensis* Tubangui from the duck in the Philippines. Recently Penner and Fried (1961) and Fried (1962) reported experimental infections of chicks with an unnamed species of Philophthalmus obtained from a marine snail, *Batillaria minima*, from Florida. In spite of successful experimental infections with this species, the fact that a marine snail is involved in the life cycle makes it improbable that domestic poultry would be found infected under natural conditions

TREMATODES OF THE RESPIRATORY SYSTEM

Several species of the family Cyclocoelidae occur in domestic fowl. These flukes are relatively large and have flattened oval or lancet-shaped bodies. The oral sucker is weakly developed or absent, and the acetabulum or ventral sucker is absent or rudimentary. The digestive tract is continuous posteriorly. The gonads are in the posterior end of the body with the ovary variously arranged with respect to the testes.

Typhlocoelum cucumerinum (Rudolphi, 1809)

Synonyms. *Typhlocoelum flavum* (Mehlis, 1831); *T. obovale* Neumann, 1909.

Description. Body oval, 6 to 15 mm. long by 2 to 7 mm. wide, yellow in color. Mouth terminal, not surrounded by an oral sucker; acetabulum absent. Intestinal tract continuous posteriorly and provided with median diverticula. Ovary and testes in posterior part of body, the latter deeply lobed. Uterus greatly convoluted, in median field. Eggs 151 to 180μ long by 85 to 90μ wide.

This fluke occurs in the trachea of wild waterfowl in the United States and in Europe; it has been reported from the domestic duck in South America.

Life history. Incompletely known, probably similar to that of *T. cymbium*.

Pathology. This fluke has been reported by Magalhães (1899) in Brazil as the cause of suffocation promptly resulting in death of some domestic ducks; the flukes were present in large numbers in the trachea and bronchi.

Typhlocoelum cymbium (Diesing, 1850)

Synonym. *Tracheophilus sisowi* Skrjabin, 1913.

Description. Body oval, 6 to 12 mm. long by 3 to 6 mm. wide (Fig. 36.4) and similar in appearance to *T. cucumerinum*, except that the testes are rounded instead of lobed. Eggs 122 to 154μ long by 63 to 81μ wide, containing miracidia at time of oviposition.

This species is not uncommon in wild waterfowl in various parts of the world, including the United States, and has been reported from the duck and goose. It occurs in the trachea, bronchi, air sacs, and infraorbital sinus.

Life history. The life history of this fluke has been ascertained by Szidat (1932) and by Stunkard (1934). The eggs, which contain miracidia when deposited, hatch on reaching water. The miracidium swims about and, on coming in contact with
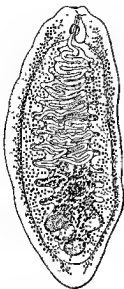
FIG. 36.4 — Typhlocoelum cymblum (= Tracheophilus sisowi). Ventral view. (From Skrjabin, 1913.)

suitable snails (*Menetus planorbis*, *Helisoma trivolvis*, *Planorbis corneus*, *Lymnaea palustris*, or *L. ovata*), penetrates the tissues and liberates a redia which is present in the body of the miracidium. The redia increases in size and gives rise to tailless cercariae which escape and become encysted in the vicinity of the redia. Birds become infected by eating the snails harboring the encysted cercariae.

Pathology. The presence of large numbers of these flukes in the larynx and trachea of birds causes death by suffocation. Light infections may cause little or no injury.

Several other cyclocoelids have been reported as parasites of poultry, namely, *Cyclocoelum mutabile* (Zeder) from the goose and turkey in Europe, Asia, and South America; *C. japonicus* Kurisu from the chicken in Japan; and *Hyptiasmus tumidus* Kossack from the goose in Europe.

In addition to flukes of the family Cyclocoelidae, Price (1937) reported *Clinostomum attenuatum* Cort (Clinostomidae), normally a parasite of bitterns, from the trachea of a chicken in Nebraska. This was apparently a case of accidental parasitism acquired through the ingestion of a tadpole or young frog containing the larval fluke.

TREMATODES OF THE DIGESTIVE SYSTEM

The digestive tract is a favorite location for flukes, and a large number of species representing many families have been reported from this organ system. Only a few of the more important of these species are discussed here.

Echinostomatidae

Flukes of this family are characterized by having a kidney-shaped collar or adoral disc armed with one or two rows of spines.

Echinostoma revolutum (Froelich, 1802)

Synonyms. *Echinostoma echinatum* (Zeder, 1803); *E. columbae* Zunker, 1925; *E. paraulum* Dietz, 1909; *E. miyagawai* Ishii, 1932; *E. cinetorchis* Ando and Ozaki, 1923.

Description. Body elongated, up to 22 mm. long (Fig. 36.5). Oral sucker surrounded by an adoral disc bearing 37 spines, 27 marginal and 5 on each ventral lobe. Acetabulum strongly developed, situated a short distance posterior to oral sucker. Testes variable in shape, one behind the other; ovary pretesticular; uterus preoraral. Eggs 94 to 126μ long by 59 to 71μ wide.

This trematode occurs in the intestine, ceca, and cloaca of a wide variety of hosts, including the chicken, duck, goose, swan, turkey, pigeon, wild waterfowl, and some mammals. It has been reported under a variety of names from practically all parts of the world.

Life history. The life cycle of *Echinostoma revolutum* has been ascertained by Johnson (1920) and by Beaver (1937). The larval stages develop in freshwater mollusks of the genera Planorbis, Helisoma, Lymnaea, Stagnicola, and Pseudosuccinea. The cercariae, which are formed in rediae and are provided with an adoral disc armed with spines as in the adult, escape and usually encyst in snails and tadpoles. The final hosts become infected through ingestion of

FIG. 36.5 — Echinostoma revolutum. Ventral view. (From Dietz, 1910.)

the infected secondary intermediate hosts.

Pathology. In most instances and in light infections, this fluke causes little injury. Heavy infections in pigeons have been reported from Europe by Zunker (1925), Krause (1925), Bolle (1925), and van Heelsbergen (1927b), and in these cases losses have resulted. Zunker stated that the small intestine of the affected birds showed hemorrhagic inflammation, and similar findings were reported by Bolle. Krause collected about 5,000 echinostomes, apparently E. revolutum, from eight pi-

geons at Rostock; the more heavily parasitized birds died, while the more lightly infected ones recovered after sickness lasting several weeks. In the cases studied by Bolle, there was a hemorrhagic diarrhea, and some of the pigeons died in an emaciated condition after being sick for 4 days. Krause reported as early symptoms the refusal of food, increased thirst, weakness in flight, and pronounced diarrhea; death occurred in 8 to 10 days following increased weakness. Van Heelsbergen in Holland also reported a severe enteritis in pigeons infected with an echinostome which appears to be E. revolutum. The birds showed atrophy of the pectoral muscles, engorged liver, and intestinal congestion, with the lumen of the gut filled with a hemorrhagic catarrhal secretion containing numerous flukes, 1,550 specimens being present in one pigeon. In the United States, Beaver (1937) reported a case of experimental infection of a pigeon which developed a bloody diarrhea 10 days after infection. At necropsy 621 flukes were recovered, the majority being in the lower duodenum and upper ileum.

Hypoderaeum conoideum (Bloch, 1782)

Synonyms. Echinostoma oxycephalum (Rudolphi, 1819); Opisthorchis pianae Galli-Valerio, 1898; Psilochasmus lecithosus Otte, 1926.

Description. Similar in size and appearance to Echinostoma revolutum (Fig. 36.6). Adoral disc poorly developed, bearing a double row of 49 short spines. Eggs 95 to 108μ long by 61 to 68μ wide.

This species occurs in the small intestine of numerous wild waterfowl and has been found in the chicken, goose, and pigeon. It has been reported from the domestic duck in the United States by Stunkard and Dunihue (1931).

Life history. Similar to that of E. revolutum, snails of the genera Lymnaea, Stagnicola, and Planorbis serving as primary intermediate hosts, and Planorbis and tadpoles serving as secondary intermediate hosts.

Pathology. Not well known. In a duck

FIG. 36.6 — Hypoderaeum conoideum. Ventral view. (From Dietz, 1910.)

FIG. 36.7 — Echinoparyphium recurvatum. (A) Entire worm. Ventral view. (B) Anterior end, (C) Encysted cercaria. (From Bittner, 1925.)

experimentally infected with 40 of these flukes, Vevers (1923) found a localized inflammation of the infected portion of the intestine, and the bird had been weak before death.

Echinoparyphium recurvatum (Linstow, 1873)

Description. Body (Fig. 36.7A) 0.7 to 4.5 mm. long, with the anterior part strongly recurved ventrally. Adoral disc (Fig. 36.7B) armed with 45 spines in a double row. General organization similar to that of Echinostoma revolutum. Eggs 108 to 120μ long by 64 to 84μ wide.

This parasite is widely distributed, having been reported from Europe, Asia, Africa, and North America. It occurs in various wild waterfowl and has been found in the duck, chicken, and pigeon in Europe, in turkey poults and chickens in the United States, and in the chicken in Mexico.

Life history. Similar to that of Echinostoma revolutum. The larval stages develop in fresh-water snails of the genera Lymnaea, Planorbis, and Viviparus. The cercariae encyst (Fig. 36.7C) in snails and tadpoles. After ingestion of infected snails and tadpoles, the flukes become mature in the small intestine of the final host, and eggs appear in the feces in 5 to 7 days.

Pathology. Van Heelsbergen (1927a) reported that in Holland infected chickens showed a severe enteritis. The parasitized birds were emaciated, anemic, and developed weakness of the legs. Annereaux (1940) in California observed in a 10-week-old turkey with 267 adult flukes in the upper portion of the small intestine a "severe inflammation of the intestinal mucosa with cecal involvements consisting of a pasty, cheeselike mass which greatly distended the organs."

Psilostomidae

Flukes of this family resemble, in general morphology, species of the Echino-

stomatidae but are not provided with a spine-bearing adoral disc.

Ribeiroia ondatrae (Price, 1931)

Synonym. *Psilostomum ondatrae* Price, 1931; *Cercaria marini* Faust and Hoffman, 1934.

Description. Elongate oval flukes measuring 1.6 to 3 mm. in length (Fig. 36.8), cuticle spiny. Oral sucker and acetabulum well developed. Esophagus with lateral diverticula. Testes in posterior end of body, ovary pretesticular. Vitellaria consisting of relatively large follicles extend ing from level of esophagus to posterior end of body. Uterus between ovary and acetabulum. Eggs 82 to 90μ long by 45 to 48μ wide

This fluke, which was originally described from the muskrat in Canada by Price (1931a), occurs in the proventriculus of several fish-eating birds, including California gull, green heron, osprey, and Cooper's hawk. It has also been reported in natural infection in the chicken in Colorado by Newsom and Stout (1933), in domestic geese in Canada by Kingscote (1951), in experimental infections in the chicken, duck, pigeon, and canary by Beaver (1939), and parakeet and pigeon by Riggin (1956).

Life history. Similar to that of the echinostomes. According to Beaver, the primary intermediate host is a fresh-water snail, *Helisoma antrosum percarinatum*, and the secondary intermediate hosts are fishes, including perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), small-mouth black bass (*Micropterus dolomieu*), pumpkin seed (*Eupomotis gibbosus*), bluegill (*Lepomis pallidus*), and bullhead (*Ameiurus*). Riggin reported the snail host in Puerto Rico to be *Australorbis glabratus* and experimental hosts for the metacercariae to be the guppy (*Lebistes reticulatus*), minnow (*Poecilia vivipara*), and tadpoles. The cercariae become encysted principally in the lateral line canal of fishes and in the cloaca of tadpoles. In the final host the flukes reach maturity in 6 to 7 days.

FIG. 36.8 – *Ribeiroia ondatrae*. Ventral view. (Newsom, Colo. St. Coll.)

Pathology. Newsom and Stout reported outbreaks of proventriculitis in two flocks of chickens in Colorado. The birds lost their appetite, stood around with their eyes closed, and gradually wasted away. Gross examination "showed a very noticeable enlargement of the proventriculus. On opening this organ there seemed to be a deep reddening around the orifices of the glands. In the more extreme cases there appeared to be a grayish exudate on the surface, simulating ulceration" (Fig. 36.9). Microscopic examination "showed that the surface of the mucous membrane was covered with a fibrinous exudate, the outer portion of which had become necrotic. Below this necrotic area was a thick zone heavily infiltrated with polymorphonuclear leukocytes. Under this, the mucous layer was quite edematous in which were scattered a few polymorphonuclear leukocytes and a few monocytes. In a few places small abscesses had formed in the lower portion of the mucous membrane." Beaver observed in experimental infections in chickens and canaries that this fluke is fairly pathogenic, each worm forming in the proventriculus a separate lesion which is a deeply eroded pit with
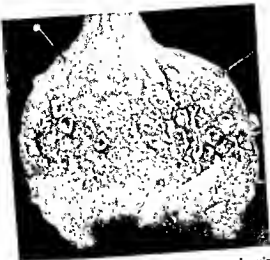
FIG. 36.9 — Proventriculus of chicken showing lesions caused by *Ribeiroia ondatrae*. (Newsam, Colo. St. Coll.)

a raised orifice surrounded by a conspicuous reddish to purple area.

Sphaeridiotrema globulus (Rudolphi, 1814)

Description. Body piriform to globular, 0.5 to 0.85 mm. long (Fig. 36.10). Suckers well developed, acetabulum massive. Genital aperture lateral, at level of posterior margin of oral sucker. Testes in posterior end of body, one dorsal to other. Ovary pretesticular; vitellaria consisting of large follicles extending from intestinal bifurcation to level of anterior margins of testes; uterus relatively short, largely preacetabular. Eggs 90 to 105μ long by 60 to 67μ wide.

This fluke occurs in the small intestine of the wild duck in Europe and North America and has been reported from the domestic duck and swan. In the United States this trematode was reported by Price (1934) as causing extensive loss among lesser scaup ducks near Washington, D.C., and it has also been found by Shaw in the domestic duck in Oregon.

Life history. As determined by Szidat (1937) in Germany, the cercaria of this fluke develops from redia in the snail *Bithynia tentaculata*. The cercariae become encysted between the shell and mantle of this snail, and birds acquire the
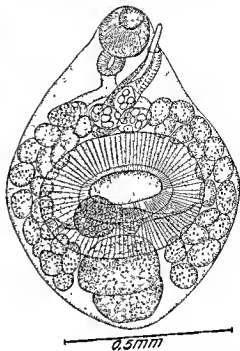
FIG. 36.10 — *Sphaeridiotrema globulus*, Ventral view. Original.

parasite through ingestion of the infected mollusks, eggs appearing in the feces 5 to 6 days later.

Pathology. This fluke produces in wild ducks a severe ulcerative enteritis. In the cases studied by Price, the small intestine, especially the lower third, showed marked congestion, hemorrhage, and ulceration, the lumen of the involved portion of the gut being filled with a cast composed largely of fibrin. Histologically the serosa, muscular, and mucous layers of the intestine showed evidence of acute hyperemia; the mucous membrane showed pronounced desquamation of epithelium, the villi being entirely denuded in most areas. There was severe ulceration in places, the ulcers frequently extending as deep as the muscularis and containing numerous flukes firmly attached by means of their powerful suckers.

Sphaeridiotrema spinoacetabulum Burns, 1961

Description. Body oval to pyriform 1.08 to 1.10 mm. long by 0.583 to 0.778 mm.
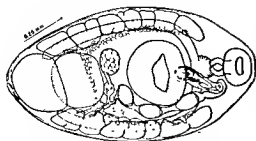
FIG. 36.11—Sphaeridiotrema spinoacetabulum. Ventral view. (From Burns, 1961.)

FIG. 36.12—Cotylurus flabelliformis. (A) Lateral view showing digestive and excretory systems. (B) Female genital system. (C) Male genital system. (From Van Haitsma, 1931.)

wide (36.11). Suckers well developed, acetabulum smaller than in *S. globulus* and armed with spines around its opening. Eggs 100 to 115μ long by 60 to 75μ wide. Other characters similar to those of *S. globulus*.

This species occurs in the ceca of ducks; so far it is known only from Oregon.

Life history. According to Burns (1961), the cercariae develop from rediae in the snail *Fluminicola virens*; they emerge from the anal opening and, upon coming in contact with this snail and *Oxytrema silicula*, encyst between the shell and mantle. About 16 days are required for the flukes to reach maturity after ingestion of encysted metacercariae.

Pathology. In infected ducklings the ceca show intense hyperemia, hemorrhage into the lumen, and marked ulceration of the mucosa. Heavy infections in young ducks may result in death.

Strigeidae

The strigeids are characterized by having the body divided by a constriction into two parts, an anterior cup-shaped portion containing the suckers and a peculiar tongue-shaped adhesive organ, and a cylindrical posterior portion containing the reproductive organs.

Cotylurus flabelliformis (Faust, 1917)

Description. Body 0.56 to 0.85 mm. long, anterior cup-shaped portion 0.20 to 0.28 mm. long, and posterior cylindrical portion 0.36 to 0.57 mm. long (Fig. 36.12). Genital aperture at posterior end of body. Eggs 100 to 112μ long by 68 to 76μ wide.

The fluke occurs in the intestine of a number of wild ducks in the United States and has been reported from the domestic duck; it has also been reared experimentally in chickens.

Life history. The cercarial and precercarial stages occur in snails of the genera Helisoma, Planorbis, Stagnicola, Lymnaea, and Physa. The cercariae, which develop in sporocysts in the snail, are fork-tailed. The cercariae that escape from the primary snail intermediate host penetrate into other snails and develop into tetracotylid larvae. When snails containing the encysted tetracotylids are ingested by a definitive host, the worms mature in 3 to 4 days.

Pathology. According to Van Haitsma (1931), *C. flabelliformis* digests away the epithelium of the intestine of the host and causes a congestion of the subepithelial tissue. The symptoms shown by infected ducks appear to vary greatly. Some of the infected ducks studied by Van Haitsma showed leg weakness, nervous twitchings of the head and wings, dyspnea, diarrhea, and irregular appetite, while others which had been given heavy doses of larvae died within a week without showing definite symptoms.

The only other strigeid reported in natural infections from poultry in the United States is *Strigea falconis meleagris* Harwood (1931). This parasite was found in viscera of turkeys at Houston, Texas, and so far as is known is of little economic importance.

A number of strigeid flukes have been

reported from poultry in other parts of the world and include *Strigea intermedia* Szidat from the duck and goose in Germany and *Cotylurus cornutus* (Rudolphi) from the duck, goose, swan, and pigeon in Europe and South America.

Brachylaemidae

Flukes of this family are characterized mainly by having the gonads in a linear series in the posterior end of the body, the ovary being situated between the testes. The genital pore is in the zone of the gonads.

Postharmostomum gallinum (Witenberg, 1923)

Synonyms: *Harmostomum* (*Postharmostomum*) *horizawai* Ozaki, 1925; *H. annamense* Railliet, 1925; *H.* (*P.*) *hawaiiensis* Guberlet, 1928.

Description. Body linguiform, 3.5 to 7.4 mm. long (Fig. 36.13). Oral sucker and acetabulum relatively well developed, the latter situated about one-third of body

FIG. 36.13—*Postharmostomum gallinum*. Ventral view. (From Skrjabin, 1924.)

length from anterior end. Intestinal ceca with wide serpentine undulations. Ovary between testes, in posterior end of body. Vitellaria lateral, extending anteriorly as far as posterior margin of acetabulum; uterus extending anteriorly as far as intestinal bifurcation. Eggs 29 to 32μ long by 18μ wide.

This trematode occurs in the ceca of the chicken, turkey, guinea fowl, and pigeon in Europe, Asia, and Africa. It has also been reported from the chicken in Hawaii and Puerto Rico.

Life history. According to Alicata (1940), the eggs contain miracidia when oviposited. When these eggs are eaten by the snail *Eulota similaris*, the eggs hatch, and the miracidia enter the liver and develop into sporocysts. The cercariae developing in the sporocysts escape and leave the body of the snail; they may reenter the same snail host or others of the same or a different species where they become encysted in the pericardial cavity. Another land snail, *Subulina octona*, has been shown to harbor the metacercariae, but it has not been determined whether this snail may also serve as a primary intermediate host. In the Orient, *Euhadra peliomphala*, *Philomycus bilineatus*, and *Eulota sieboldiana minor* have been reported as capable of serving as secondary intermediate hosts.

Pathology. So far as known, these flukes cause little or no injury to their bird hosts. It is possible that in extreme cases of heavy infection, some irritation or inflammation of the ceca might result from the presence of the worms.

Notocotylidae

The notocotylids are small to medium-sized monostomes. The ventral surface is usually provided with rows of glands or ridges (absent in *Paramonostomum*). There is no pharynx, and the tips of the intestinal ceca pass between the testes, which are located in the posterior part of the body. The eggs are small and are provided with a long slender filament at each pole.

Notocotylus imbricatus (Looss, 1893)

Synonyms. *Notocotylus seineti* Fuhrmann, 1919; *N. urbanensis* Harrah, 1922, in part; *N. intestinalis* Tubangui, 1932.

Description. Body elongate, oval, 2 to 4 mm. long (Fig. 36.14A and B). Ventral surface with 3 linear rows of glands, 12 to 16 in median row and 12 to 17 in each lateral row. Eggs (Fig. 36.14C) 17 to 20μ long by 9 to 12μ wide.

This species is perhaps the widest distributed of the notocotylids and occurs in Europe, Asia, and North America. It has been reported from ducks and numerous wild waterfowl and has been reared experimentally in the chicken. According to Harwood (1939), this fluke has been collected from domestic ducks in Oregon and New York.

Life history. The larval stages develop in the livers of snails of the genera Bithynia, Lymnaea, and Physa. When the cercariae escape from the intermediate host, they encyst on the shell of the snail or on other objects. When the cysts are ingested by suitable bird hosts, the young flukes are liberated and develop to maturity in the rectum and ceca.

Pathology. Flukes of this genus produce little injury to their hosts. It is possible that if present in large numbers they may cause some inflammation of the rectum and ceca.

Other species of Notocotylus reported from poultry are *N. attenuatus* (Rudolphi) from the duck, goose, turkey, and chicken in Europe and Asia; *N. ephemera* (Nitzsch) from the chicken and duck in Europe; *N. chionis* Baylis from the goose in Europe; and *N. aegyptiacus* Odhner from the duck in Africa (Egypt).

Catatropis verrucosa (Froelich), a notocotylid having a glandular keel or ridge instead of a median row of glands, occurs in the duck, goose, and chicken in Europe, and chicken in the United States. *Paramonostomum alveatum* (Mehlis) and *P. parvum* Stunkard and Dunihue, species without ventral glands, occur in domestic ducks in Europe and North America, respectively.

Paramphistomidae

This family comprises flukes having the acetabulum or ventral sucker situated at the posterior end of the body. Only one species occurs in poultry.

Zygocotyle lunata (Diesing, 1836)

Synonym. *Zygocotyle ceratosa* Stunkard, 1916.

Description. Body ovate, up to 9 mm. long (Fig. 36.15). Oral sucker subventral, provided with two evaginations or pouches. Acetabulum terminal, large, with its posterior margin provided with a flap terminating on each side in a conclike projection. Eggs 124 to 153μ long by 72 to 96μ wide.

This fluke occurs in the ceca of a number of wild waterfowl and has been reported by Price (1928) from the goose in the United States and by Caballero (1941) from the chicken in Mexico; it has also been reared experimentally in domestic ducks by Willey (1941).

Life history. The life history of this form
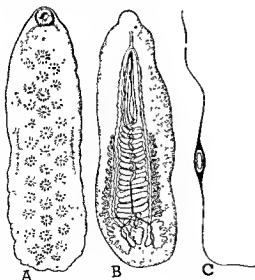
FIG. 36.14 — Notocotylus imbricatus (= N. seineti). (A) Ventral view, showing glands. (B) Dorsal view, showing internal organization. (C) Egg. (From Fuhrmann, 1919.)

FIG. 36.15 — Zygocotyle lunata. Ventral view.
(Willey, New York Univ.)

has been studied in detail by Willey. The larval stages develop in the snail *Helisoma antrosum*. The cercariae which develop in rediae escape from the snail intermediate host and encyst on such objects as pond weeds and the shells of snails; infection of the final or definitive host occurs when the cysts are eaten. The flukes mature and give off eggs in about 6 weeks.

Pathology. So far as known, these flukes produce no appreciable injury to their bird hosts.

TREMATODES OF THE LIVER

The trematodes of the liver of poultry belong for the most part to the family Opisthorchiidae. They are semitransparent, usually elongate flukes, and occur in the bile ducts. Only one opisthorchiid has been reported as a parasite of poultry in the United States. This form, *Amphimerus* sp., was recorded by Price (1931b) from a turkey from North Dakota. The liver of this bird showed marked distention of the bile ducts and extensive pressure atrophy of the liver parenchyma.

Several opisthorchiids have been reported from ducks in various parts of the

world and include *Opisthorchis simulans* (Looss) from Europe; *O. longissimus* (Linstow) from Russia; and *Amphimerus anatis* (Yamaguti) from Japan. Closely related flukes of the genus Metorchis occur in ducks in Europe and elsewhere.

TREMATODES OF THE URINARY SYSTEM

The trematodes occurring in the kidneys of poultry belong to the family Eucotylidae. These flukes lack a ventral sucker and a cirrus pouch and have a long tortuous uterus filling the greater part of the pre- and post-testicular fields.

Tamerlania bragai dos Santos, 1934

Description. Body elongate, flat, up to 3 mm. long (Fig. 36.16). Oral sucker subterminal, acetabulum present, minute, according to Stunkard (1945). Pharynx relatively large; esophagus absent; intestinal ceca extending to and uniting near posterior end of body. Testes side by side in middle of body; ovary more or less triangular, immediately pretesticular. Vitel-
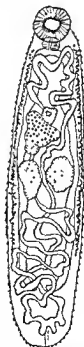
FIG. 36.16 — Tamerlania bragai. Ventral view.
(From dos Santos, 1934.)

laria lateral, extending from level of pharynx to about one-fourth of body length from posterior end. Uterus convoluted, pre- and post-testicular. Eggs 31μ long by 13μ wide.

This fluke is found in the kidneys and ureters of pigeons in Brazil, Puerto Rico, and the Philippine Islands; it has also been reported from the chicken in Brazil

Life history. Maldonado (1945) reported that the intermediate host is a land snail, Subulina octona; the larval cycle is completed in about a month. Birds become infected upon ingestion of infected snails containing encysted metacercariae Eggs are recoverable in the urine and excreta 23 days after infection.

Pathology. According to dos Santos (1931), the presence of the flukes in the kidney caused distention of the collecting tubules and a thickening of their walls, the lumen of the tubules being filled with amorphous and crystallized detritus. The parenchyma of the kidney showed extensive cellular infiltration, but the cortex was rarely involved. Maldonado and Hoffman (1911) noted similar changes in pigeons in Puerto Rico but were of the opinion that the parasites caused no ill effect, since birds that were kept in cages for several months appeared unaffected by the parasites.

TREMATODES OF THE REPRODUCTIVE SYSTEM

The flukes of the reproductive system belong to the Plagiorchiidae, a family which is characterized by having the ascending and descending limbs of the uterus passing between the testes. Several representatives of this family occur in American poultry, the most important of which is discussed below.

Prosthogonimus macrorchis Macy, 1934

Description. Body piriform in outline, 5.26 to 7.56 mm. long (Fig. 36 17); cuticula spiny. Intestinal ceca simple, extending to near posterior end of body. Genital pore at anterior end of body, slightly to left of oral sucker; testes oval,

FIG 36 17 – Prosthogonimus macrorchis. Complete worm from oviduct of chicken. (Macy, College of St Thomas, St. Paul, Minn.)

opposite each other and about one-third of body length from posterior end. Ovary greatly lobulated, immediately posterior to acetabulum, vitellaria lateral, extending from acetabulum to testes; uterus with numerous coils in post testicular part of the body Eggs 28μ long by 16μ wide, with spine of variable shape and length at antopercular pole.

This fluke occurs in the bursa Fabricii and oviduct of the duck, chicken, and other birds in the United States; it is particularly common in the lake region of Michigan and Minnesota.

Life history. According to Macy (1934), "The sporocyst, found in the 'liver' of Amnicola limosa porata, produces the cercaria directly, there being no redia stage. The cercaria swims away from the snail host, and, if it is drawn into the anal opening of a suitable species of dragonfly naiad by the breathing movements of such a host, the tail of the cercaria is lost and the metacercaria thus formed makes its way to the muscle of the naiad, where it increases to about five times its original size. A thick wall with an outer radially-striated and an inner homo-
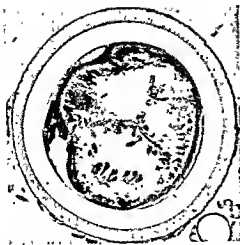
FIG. 36.18 — Section through metacercaria of *Prosthogonimus macrochis* from abdomen of a dragonfly. (Macy, College of St. Thomas, St. Paul, Minn.)

geneous layer (Fig. 36.18) now forms about the metacercaria, and the cyst usually comes to lie in the body cavity of the host. In the event the infected dragonfly naiad or adult is eaten by a suitable avian definitive host, the wall is digested off the cyst as it passes down the digestive tract of the bird. The worm then makes its way down the intestine to the cloaca and then to the bursa Fabricii or to the oviduct, where it develops into the mature trematode. Embryonated eggs produced by the fluke leave the host by way of the cloacal opening, and if they reach a lake inhabited by *Amnicola limosa* the latter become infected and sporocysts and cercariae develop. The important dragonfly hosts of *P. macrochis* belong to the genera *Leucorrhinia*, *Tetragoneuria*, and *Epicordulia*.

Pathology. The lesions and symptoms caused by species of *Prosthogonimus* in Europe have been described by Hieronymi and Szidat (1921). Reinhardt (1922), Seifried (1923), de Blieck and van Heelsbergen (1922), and others, and in the United States by Kotlán and Chandler (1925) and Macy (1934). The disease in American fowl caused by *Prosthogonimus macrochis* is essentially the same as that in Europe caused by *P. pellucidus*. Af-

fected birds lose their normal activity and appetite, and there is a pronounced dropping off in egg production. The eggs that are produced frequently have very thin shells or no shells. On necropsy there may be extreme emaciation and anemia, and an adhesive peritonitis. The intestines may show pronounced hyperemia and be covered with a fibrinous exudate. The oviduct may show similar changes, be distended, and contain considerable exudate and egg material. In some cases there may be a rupture of the oviduct with the secretions, albumen and yolk material, present in the body cavity. Flukes are present in the oviduct and in the egg material, as well as in the abdominal cavity in the case of oviduct rupture. In some instances the peritonitis may be so pronounced as to be detected in the dead and unopened birds by the bluish-red color of the abdominal wall. These lesions, as well as the laying of thin-shelled eggs or of eggs with no shells, may be due to other causes, but it seems to be an established fact that the flukes may be a contributory cause, if not the actual cause, of this condition in many instances. At any rate, if such conditions are encountered in areas where dragonflies are breeding, such as in the lake regions of the country, prosthogonimiasis should be suspected.

TREMATODES OF THE CIRCULATORY SYSTEM

All of the trematodes living in the circulatory system of birds belong to the family Schistosomatidae and are characterized by having the sexes separate. Several species, namely, *Bilharziella polonica* (Kowalewski), *Pseudobilharziella yokogawai* (Oiso), *Dendritobilharzia pulverulenta* (Braun), *Trichobilharzia ocellata* (La Valette), and *Gigantobilharzia monocotylea* Szidat occur in domestic waterfowl in Europe and elsewhere; but none of this group is known to occur in this country. Several schistosomes are known from wild waterfowl in North America, and some of them will probably be found

capable of infecting poultry. In spite of the fact that the blood flukes are serious parasites of man, those infecting poultry do not seem to cause comparable injury to their bird hosts. Szidat (1929) reported that, in infections with *Bilharziella polonica*, the eggs of the fluke in the intestinal wall caused slight connective tissue proliferation and some leukocytic infiltration; in infections with *P. yokogawai*, Oiso (1927) noted pathological changes in the liver and intestine and arrested growth of the bird host.

TREATMENT OF POULTRY TREMATODIASES

Owing to the fact that trematode infections of poultry are rarely diagnosed ante mortem, practically nothing is known concerning effective treatment for their removal. Medicinal treatment would appear to be of no value for the removal of the skin fluke, *Collyriclum faba*, surgical incision and mechanical removal of the worms seeming to be the rational procedure in such infections.

Flukes occurring in the respiratory system, particularly of the nasal passages, trachea, and bronchi, might possibly be removed by inhalations of powdered drugs having vermicidal properties. The most promising of such drugs is barium antimonyl tartrate, which is highly effective against the poultry gapeworm. The method of administration of this drug is discussed on page 996.

For trematodes occurring in the digestive tract, carbon tetrachloride in doses of 1 to 3 cc., depending on the kind and size of the bird, might be tried. In case the flukes are in the proventriculus or in the upper part of the intestine, the drug may be introduced directly into the former organ by means of a syringe and rubber catheter. For flukes in the lower part of the intestine or in the ceca, 2 to 5 cc. of carbon tetrachloride in three to four times its volume of a bland oil, such as mineral oil or cottonseed oil, administered by rectal injection, would probably prove effective.

In infections with the oviduct fluke Prosthogonimus, carbon tetrachloride is again the most promising treatment. Schmid (1930) reported the administration to a hen of 1.5 cc. of this drug in an equal amount of flour paste. On the following day the bird received 1 cc. of the drug in 8 cc. of the paste, and on the third day 1.7 cc. of the drug in the same amount of the paste. At this time no more Prosthogonimus eggs could be found in the feces. On the day following the last treatment, a mass of egg yolk containing nine of the flukes was found in the cage. The bird was then killed, and in the oviduct were found two small egg concretions in which several flukes were lying, and other flukes were imbedded in collections of mucus; all of the flukes apparently were dead. Other birds in the flock were treated, but the results were inconclusive.

No drug treatment of value is known for the destruction of fluke parasites of the excretory and circulatory systems.

CONTROL OF POULTRY TREMATODES

In view of the fact that all of the trematode parasites of poultry require at least one snail intermediate host, measures for the prevention of fluke infections must be directed toward control or eradication of these mollusks, or to keeping poultry away from areas where the parasites may be acquired. The latter is the easier and perhaps the most certain method of preventing the birds from acquiring trematode infections, and consists of selecting areas for poultry raising that are as far removed as possible from streams or swampy places, or by fencing to keep the birds from ranging over such areas.

The control of the snail intermediate hosts may be accomplished either by draining the low, marshy places or by the use of chemicals that are toxic to the snails. In the case of swampy areas, drainage, either by means of open ditches or by the

use of agricultural tile, will lower the water table to a point where there is insufficient surface moisture to enable the snails to propagate. If drainage should be too expensive or otherwise impractical, the snails may be destroyed by dusting the area with powdered copper sulfate or bluestone. The copper sulfate should be mixed with a carrier, such as fine sand or land plaster in the proportion of 1 part of the chemical to 4 to 8 parts of the carrier, and spread either by broadcasting by hand or by the use of hand or power dusters. For destroying snails in ponds and small lakes, the powdered copper sulfate may be used as in the case of marshes. The chemical should be spread along the banks and in the water near the shore, as most of the snails will be found in these locations.

For destroying snails in streams, burlap sacks containing large crystals of copper sulfate may be placed in the streams at the uppermost part of the section to be treated in an amount sufficient to give a concentration of 1 part of the chemical to about 500,000 parts of water. The amount of the chemical necessary may be determined by ascertaining the cross-section area of the stream and multiplying by the velocity in order to get the flow in cubic feet per second. This result multiplied by 12, which is the amount in pounds of copper sulfate necessary to give a concentration of the chemical of 1 to 500,000 for a 24-hour period, equals the amount of copper sulfate needed for the treatment. For example, if a stream is 3 feet wide and 1 foot deep and the velocity is 2 feet per second, the flow is 6 cubic feet per second; this result times 12 equals 72, or the number of pounds of copper sulfate necessary for one treatment. This concentration of the chemical will kill most snails but is not injurious to livestock; it may kill some fish and will destroy algae and moss.

In some instances, especially with Prosthogonimus, where the fluke is acquired through the ingestion of dragonflies, keeping poultry away from the shores of ponds or lakes in the mornings when these insects are inactive is recommended.

REFERENCES

Alicata, J. E.: 1940. The life cycle of *Postharmostomum gallinum*, the cecal fluke of poultry. Jour. Parasit. 26:135.

———: 1962. Life cycle and developmental stages of *Philophthalmus gralli* in the intermediate and final hosts. Jour. Parasit. 48:47.

——— and Noda, K.: 1960. Observations on the life history of *Philophthalmus*, a species of eye fluke of birds in Hawaii. Libro Homenaje Caballero y Caballero, p. 67.

Annereaux, R. F.: 1940. A note on *Echinoparyphium recurvatum* (von Linstow) parasitic in California turkeys. Jour. Am. Vet. Med. Assn. 96:62.

Beaver, P. C.: 1937. Experimental studies on *Echinostoma revolutum* (Froelich), a fluke from birds and mammals. Ill. Biol. Monograph 15, 96 pp.

———: 1939. The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda. Psilostomidae). Jour. Parasit. 25:383.

Bolle, W.: 1925. Über einen Tanbenrematoden aus der Gattung Echinostomum. Deutsch. tierärztl. Wochenschr. 33:529.

Burns, William C.: 1961. The life history of *Sphaeridiotrema spinoacetabulum* sp. n. (Trematoda:Psilostomidae) from the ceca of ducks. Jour. Parasit. 47:933.

Caballero y C., Eduardo: 1941. Parasitismo en *Gallus gallus* L. originado por *Zygocotyle lunatum* en la region de Lerma. III An. Inst. Biol., Univ. Nac. Mexico 12:423.

de Blieck, L., and van Heelsbergen, T.: 1922. Trematoden als oorzaak van eileider-ontsteking en het leggen van windeieren. Tijdschr. Diergeneesk. 49:536.

dos Santos, V.: 1955. Monostomose renal dos aves domesticas. (Portuguese text. French and English summaries) Rev. Dept. Nac. Prod. Anim. 1:203.

Fried, Bernard: 1962. Growth of *Philophthalmus* sp. (Trematoda) in the eyes of chicks. Jour. Parasit. 48:595.

Harwood, P. D.: 1939. *Strigea falcons meleagris*, n. var. Jour. Parasit. 18:51.

———: 1939. Notes on Tennessee helminths. IV. North American trematodes of the subfamily Notocotylinae. Jour. Tenn. Acad. Sci. 14:421.

Hieronymi, E., and Szidat, L.: 1921. Über eine neue Hühnernematode, bedingt durch *Prosthogonimus intercalandus*, n. spec. Zentralbl. f. Bakt., I. Orig. 86:256.

Jegen, G.: 1917. *Collyriclum faba* (Bremser) Kossack. Ein Parasit der Singvögel, sein Bau und seine Lebensgeschichte. Zeitschr. wiss. Zool. 117:460.

Johnson, J. C.: 1920. The life cycle of *Echinostoma revolutum* (Froelich). Univ. Calif. Publ. in Zool. 19:335.

Kingscote, A. A.: 1951. A note on *Ribeiroia ondatrae* Price 1931 (Trematoda). Jour. Parasit. 37:324.

Kotlán, A., and Chandler, W. L.: 1925. A newly recognized fluke disease (prosthogonimiasis) of fowls in the United States. Jour. Am. Vet. Med. Assn. 67:756.

Krause, C.: 1925. Gehäuftes Sterben bei Tauben durch Echinostomiden. Berliner tierärztl. Wochenschr. 41:202.

Macy, R. W.: 1934. Studies on the taxonomy, morphology, and biology of *Prosthogonimus macrorchis* Macy, a common oviduct fluke of domestic fowls in North America. Univ. Minn. Agr. Exper. Sta., Tech. Bul. 98.

Magalhaes, P. S.: 1899 Note d'helminthologie brésilienne. 9. Monostomose suffocante des canards. Arch. Parasit. 2:258.

Maldonado, J. F.: 1945. The life cycle of *Tamerlania bragai*, Santos 1934 (Eucotylidae), a kidney fluke of domestic pigeons. Jour. Parasit. 31:306.

———, and Hoffman, W. A.: 1941. *Tamerlania bragai*, a parasite of pigeons in Puerto Rico. Jour. Parasit. 27:91

Marotel, G.: 1926 Une nouvelle maladie parasitaire, la monostomidose cutanée du dindon. Rev. vét. méd. 78:725.

Newsom, I. E., and Stout, E. N.: 1933. Proventriculitis in chickens due to flukes. Vet. Med. 28:462.

Oiso, T.: 1927. On a new species of avian Schistosoma developing in the portal vein of the duck, and investigations of its life-history (Japanese text; English summary) Taiwan Igakkwai Zasshi (270):818.

Penner, L. R., and Fried, B.: 1961. Studies on ocular trematodosis 1 Marine acquired philophthalmiasis. Jour. Parasit. 47(supp):51.

Price, E. W.: 1928. The host relationship of the trematode genus *Zygocotyle*. Jour. Agr. Res. 36:911.

———: 1931a. Four new species of trematode worms from the muskrat, *Ondatra zibethica*, with a key to the trematode parasites of the muskrat Proc. U.S. Nat. Mus. (2870), 79, Art. 4:1–13.

———: 1931b. Trematode of genus Amphimerus in liver of domestic turkey. Jour. Parasit. 18:51.

———: 1934 Losses among wild ducks due to infestation with *Sphaeridiotrema globulus* (Rudolphi) (Trematoda; Psilostomidae) Proc. Helminth. Soc. Wash. 1:31.

———: 1937 A note on the occurrence of a trematode of the genus *Clinostomum* in a chicken. No. Am. Vet. 18 (April):33.

Reinhardt, R.: 1922 beschreibhaft auftretende Eilesterentzündungen bei Hühnern durch Invasion von *Prosthogonimus intercalandus*. Berliner tierärztl. Wochenschr. 38:384.

Riggin, G. T.: 1956. A note on *Ribeiroia ondatrae* (Price, 1931) in Puerto Rico. Proc. Helminth. Soc. Wash. 23,28.

Riley, W. A.: 1931. *Collyriclum faba* as a parasite of poultry. Poultry Sci. 10:204.

———, and Kernkamp, H. C. H.: 1924. Flukes of the genus Collyriclum as parasites of turkeys and chickens. Jour. Am. Vet. Med. Assn. 64:591.

Schmid, R.: 1930. Beitrag zur Geflügelparasiten-Behandlung. Tierärztl. Rundschau 36:513.

Seifried, O.: 1923. Durch Invasion von Trematoden (Prosthogonimus-Arten) verursachte seuchenhaft auftretende und tödlich verlaufende Eileiter-Erkrankungen bei Hühnern in Mecklenburg. Deutsch. tierärztl. Wochenschr. 31 541.

Stunkard, H. W.: 1934. The life history of *Typhlocoelum cymbium* (Diesing, 1850) Kossack, 1911 (Trematoda, Cyclocoelidae). A contribution to the phylogeny of the monostomes. Bul. Soc. zool. France 59:447.

———: 1945. The morphology of *Tamerlania bragai* dos Santos 1934. Jour. Parasit. 31:301.

———, and Dunihue, F. W.: 1931. Notes on the trematodes from a Long Island duck with description of a new species. Biol. Bul. 60:179

Szidat, L.: 1929. Die Parasiten der Hausgeflügels. 3. *Bilharziella polonica* Kow., ein im Blut schmarotzender Trematode unserer Enten, seine Entwicklung und Uebertragung. Arch. Geflügelk. 3:78.

———: 1932. Zur Entwicklungsgeschichte der Cyclocoeliden. Die Lebenszyklus von *Tracheophilus sisowi* Skrj. 1923. Zool. An 100:205.

———: 1937. Über die Entwicklungsgeschichte von *Sphaeridiotrema globulus* Rud. 1814 und die stellung der Psilostomidae Odhner an naturlichen System. 1. Die Entwicklungsgeschichte von *Sphaeridiotrema globulus* Rud. Zeitschr. Parasitenk. 9.529.

Tyzzer, E. E.: 1918. A monostome of the genus Collyriclum occurring in the European sparrow, with observations on the development of the ovum Jour. Med. Res. 38, n.s. 33.267.

Van Haitsma, J. P.: 1931. Studies on the trematode family Strigeidae (Holostomidae). No. XXII. *Cotylurus flabelliformis* (Faust) and its life-history. Paper Mich. Acad. Sci. Arts and Letters 13:447.

van Heelsbergen, T.: 1927a. Echinostomiasis bij kippen door Echinoparyphium. Tijdschr. Diergeneesk. 54:413.

———: 1927b. Echinostomiasis bij de duif door Echinostoma. Tijdschr. Diergeneesk. 54:414.
Vevers, G. M.: 1923. Observations on the life-histories of *Hypodaeum* [*sic*] *conoideum* (Bloch) and *Echinostomum revolutum* (Froel.): Trematode parasites of the domestic duck. Ann. Appl. Biol. 10:134.
West, A. Fred: 1961. Studies on the biology of *Philophthalmus gralli* Mathis and Léger, 1910 (Trematoda:Digenea). Amer. Mid. Nat. 66:363.
Willey, C. H.: 1941. The life history and bionomics of the trematode, *Zygocotyle lunata* (Paramphistomidae). Zoologica 26.65.
Zunker, M.: 1925. *Echinostoma columbae* n. sp., ein neuer Parasit der Haustaube. Berliner tierärztl. Wochenschr. 41:483.

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37

Protozoa

COCCIDIOSIS OF THE CHICKEN

Coccidiosis is a general term applied to infection with one or more of the many species belonging to the Coccidia, a subdivision of the great protozoan class SPOROZOA, all of whose representatives are parasitic and devoid of specialized organelles of locomotion in the vegetative stages. There are, however, as Tyzzer (1932) has emphasized, as many kinds of coccidiosis as there are species of coccidia, each with its characteristic symptoms. All known types in chickens involve the digestive tract, whose cells are penetrated by the parasites. While coccidiosis is cosmopolitan and occurs in practically all kinds of birds, the problem is simplified somewhat by the fact that the parasites are host-specific; that is, each species occurs in a single species of host or limited group of

closely related hosts. In the latter case, one particular species seems to be the optimum host for the parasite. However, a particular bird host may harbor more than one species of coccidia. Thus, while the problem of identifying species of coccidia is simplified by the host limitations of these parasites, it is, on the other hand, complicated by the possibility of occurrence of multiple species in a single host species.

As a group, the coccidioses of chickens are of more economic importance than those of any other domesticated bird. Brackett and Bliznick (1950) have documented morbidity and mortality data for the species of Eimeria parasitizing chickens and have submitted evidence that losses are more far-reaching than generally recognized. Turkeys, ducks, and guinea fowls suffer less than do chickens from coccidial infection, though under certain conditions the infection may become serious in these birds. There are also on record disastrous outbreaks of renal coccidiosis in geese.

Pheasants and quail, when raised in captivity, frequently suffer serious losses.

Taxonomic relationships. Of the many known genera of the Coccidia there are but two of importance: Eimeria and Isospora. They are readily distinguished on the basis of the development of their terminal stages, the oocysts, subsequent to passage by the host. The freshly passed oocysts of both genera consist of little more than a compound wall and a rounded mass of nucleated protoplasm separated by a jelly-like material. In the presence of moisture and oxygen there characteristically develop, from the protoplasmic mass of Eimeria, four spores, or sporocysts, each containing two more or less banana-shaped sporozoites (Fig. 37.1). The matured cyst of Isospora contains but two spores, each holding four sporozoites. The net result in the case of both genera is the production of eight sporozoites inside each oocyst. Incidentally, sporozoite formation is considered to represent the final phase of the life cycle.

The distribution of the two genera among the orders of birds has been worked out by Boughton (1937a), Boughton, Boughton, and Volk (1938), Boughton and Volk (1938), and Becker (1956) who along with later workers report the occurrence of species of Eimeria in twelve orders of birds. Species of Isospora have been located in eleven orders of birds. It is likely that when sufficient numbers of hosts have been examined, species of Eimeria and/or Isospora will be found in birds of most orders. Our interest is limited mostly to the genus Eimeria, which commonly occurs in barnyard fowls and pigeons.

The life cycle. When a viable matured or sporulated oocyst of the genus Eimeria is ingested by a bird of a suitable species, eight sporozoites, whose development has been previously discussed, escape from the enclosing spore and oocyst in the intestine of the new host and invade epithelial cells of the mucosa. Ordinarily the infective stages are ingested with food or drink, and the excystation process is facilitated by the

1—Micropyle.
2—Polar inclusion.
3—Oocyst jelly.
4—Endomembrane of cyst wall.
5—Middle or "granular layer" of cyst wall.
6—Exomembrane of cyst wall.
7—Sporocyst or envelope of a spore.
8—Oocystic or extra-residual body.
9—Stieda body of spore.
10—Refractile globule of sporozoite.
11—Sporozoite.
12—Sporocystic or intra-residual body
13—Nucleus of sporozoite.
14—Small refractile globule at more attenuated end of sporozoite.

FIG. 37.1 — Diagrammatic representation of a mature oocyst of the genus Eimeria. (From Becker.)

body temperature of the bird and the action of digestive juices. Pratt (1937) and Goodrich (1944) working with *E tenella*, Itagaki (1954) with a mixture of oocysts principally *E. tenella*, Doran and Farr (1962) with *E. acervulina*, and Farr and Doran (1962) with *E. acervulina* and *E. tenella* of chickens and *E. meleagrimitis* and *E. gallopavonis* of turkeys observed excystation as indicated by liberated sporocysts and sporozoites in the digestive tracts of birds inoculated *per os*. They all concluded that the oocyst walls were ruptured before the sporozoites escaped from the sporocysts. Pratt (1937) and Itagaki (1954) observed free spores and sporozoites in the crop of chicks. Doran and Farr (1962) and Farr and Doran (1962) reported that oocysts were apparently unchanged in the crop, that a high percentage were broken in the gizzard and the liberated sporocysts passed on into the intestine, where in the presence of bile and pancreatic juice the sporozoites were activated to escape.

That other factors may be operating was demonstrated by Sharma and Reid (1962) and Davies and Joyner (1962) who succeeded in producing infection in appropriate portions of the intestinal tract following subcutaneous, intravenous and intraperitoneal inoculation of sporulated oocysts of *E. tenella* and other species of chicken coccidia. These infections were usually much lighter than those following oral inoculation but according to Davies and Joyner (1962) some were severe enough to cause death. (See also Horton Smith and Long, 1963.)

Levine (1942) and Ikeda (1955) failed to obtain infections in chickens fed sporulated oocysts when the pancreatic ducts were ligated. Ikeda (1955) demonstrated that infection could be produced if trypsin was fed along with the oocysts.

Gill and Ray (1954b) and Ikeda (1960) reported *in vitro* excystation from intact *E. tenella* oocysts using preparations of trypsin or pancreatin. On the other hand all attempts by Pratt (1937), Goodrich (1944), Itagaki and Tsubokura (1953),

Itagaki (1954), and Doran and Farr (1962) to excyst sporozoites *in vitro* from intact oocysts of chicken coccidia, using various tissue extracts, trypsin, and other commercial preparations, were unsuccessful. Itagaki (1954) reported that excystation was solely due to mechanical rupture of oocysts and sporocysts. Goodrich (1944) obtained excystation of sporozoites from liberated sporocysts using artificial mixtures containing trypsin. Doran and Farr (1962) found that low percentages of sporozoites could be excysted from free sporozoites when placed in various pancreatic preparations. In the presence of bile or bile salts the action of these preparations was greatly increased. Bile alone had no effect. These authors showed that trypsin was one of the enzymes involved, but that it was not the only one.

The presence of a sporozoite in the epithelial cell, or of the first generation trophozoite developing from it, is betrayed by an eosinophilic globule (an inclusion typical of sporozoites) observable in thin, stained sections. The young trophozoite, or schizont, is usually an ovoid or rounded body enclosing a nucleus in addition to the aforementioned globule. Growth of the schizont is accompanied by repeated binary divisions of the nuclear material so that it comes to possess a considerable number of nuclei by the time growth ceases (Fig. 37.2). The cytoplasm segments about the nuclei so that there are produced about as many first-generation merozoites as there were nuclei. Merozoites usually become sickle- or banana-shaped. Before their release from the host cell they are recognizable as a clump with the individuals lying more or less parallel like the sections of an orange. The process just described, wherein a considerable number of merozoites are produced through asexual reproduction, is known as schizogony. In most species of coccidia, as many of the first crop of merozoites as can do so enter other epithelial cells, and the process is repeated in a general way. So far as is now definitely known, the different generations of schizonts within any one species are

LIFE CYCLE OF THE CHICKEN COCCIDIUM,
EIMERIA TENELLA

FIG. 37.2 — Life cycle of E. tenella. (After Levine, 1961.)

characteristic. They differ in size, shape, number of merozoites produced, and in their effect upon the form, location, and activities of the parasitized host cell.

After several repetitions of the process of schizogony, there appears a generation of merozoites that enter epithelial cells to develop into sexual phases, or gametocytes. The latter are, in their younger stages, rounded bodies, but as growth proceeds it becomes evident that they are of two sorts: (1) males, or microgametocytes, in which growth is accompanied by many nuclear divisions, and (2) females, or macrogametocytes, which also grow but retain a single nucleus. From the microgametocyte there may develop as many microgametes as nuclei, each of which is a minute flagel-

lated body Fertilization is accomplished by the penetration of the microgamete into the macrogamete (a matured macrogametocyte) through a micropyle. The resulting zygote secretes a wall about itself, a process in which certain cytoplasmic granules are involved, and it is known as an oocyst (Pattillo and Becker, 1955).

After the oocysts are discharged in the feces of the bird the zygote divides into four sporoblasts. Each sporoblast transforms itself into a sporocyst containing two sporozoites and, usually, an intraresidual body. The time required for completion of sporulation is dependent upon temperature and oxygen supply. Edgar (1954, 1955a) reported that the optimum temperature for rapid sporulation of *E tenella* and five other species of chicken coccidia varied between 28° and 29° C Sporulation *E. tenella* was slow and poor at 8° and 37° C. According to Duncan (1959) oocysts of the pigeon coccidium *E labbeana* sporulated slowly under anaerobic conditions Wilson and Fairbairn (1961) stated that sporulation of *E. acervulina* oocysts did not occur anaerobically. (See also Ellis, 1938b, Itagaki, 1952; Long, 1959; Smith and Herrick, 1944; Schwalbach, 1961b)

Periodicity. Boughton in 1933 discovered diurnal gametic periodicity in infections of the English sparrow with Isospora. The oocysts appeared in the bird's droppings from 3 P.M. to 8 P.M each day. They commenced to appear in small numbers at the beginning of this period, reached a peak, and declined again to small numbers at the end of the period. It was concluded that the periodicity of oocyst production was affected, at least to a certain extent, by the metabolism of the host, as it was regulated by the responses of the bird to light and darkness. (Cf. Schwalbach, 1960, 1961a and b). Levine (1942a) studied the periodicity of oocyst discharge in *E. necatrix*, *E. hagani*, *E. maxima*, *E. mitis*, and *E. praecox* infections of chickens. In all cases except *E. necatrix* there was a tendency for the peak of oocyst discharge to occur during the 6 hours from 3 P.M. to 9 P.M., while in necatrix infections the highest

oocyst elimination took place between 9 P.M. and 9 A.M. The latter phenomena may be due to the fact that necatrix oocysts develop in the ceca, which do not discharge their content regularly.

Etiology. There are eight valid species of coccidia of the genus Eimeria known to occur naturally in chickens. Wenyonella was found in chickens by Ray (1945) (see page 1070). Since it is practically impossible to present anything like a complete description of the nine species without involving the effect on the host, it is recommended that the section on pathogenicity be read in connection with this one.

Table 37.1 shows that the oocysts of the eight species of Eimeria differ in respect to size, shape, and sporulation time (see Johnson, 1938; Tyzzer, 1929; Becker, Zimmermann, and Pattillo, 1955; Becker, Jessen, Pattillo, and Van Doorninck, 1956; Becker, Zimmermann, Pattillo, and Farmer, 1956). Nevertheless, it is most difficult to distinguish the species on the basis of oocyst characteristics alone, save that those of *E. maxima* can usually be readily identified by their larger size, together with their egg shape and rough walls. The minimal times that intervene between the feeding of sporulated oocysts and recovery of the next generation of oocysts in the feces are, according to Edgar (1955a), about 97 hours for *Eimeria acervulina*, 99 hours for *E. mitis*, 120 hours for *E. brunetti*, 123 hours for *E. maxima*, and 138 hours for *E. tenella*, *E. necatrix*, and *E. hagani*. Numbers discharged are scanty at first appearance but increase rapidly during the subsequent day or two until they reach a peak, after which they decline rapidly. The region of the intestine parasitized, the position of the parasites in the intestinal mucosa, the macroscopic lesions, and the clinical type are also important characters that help in the identification of species. Tyzzer and Levine have also used the cross-immunity test to advantage.

Ordinarily it is difficult to identify the species on the basis of the morphology of the asexual stages in the intestinal wall

TABLE 37.1

CHARACTERS FOR THE SEPARATION OF THE EIGHT SPECIES OF EIMERIA OCCURRING IN CHICKENS*

Characters	E. tenella	E. mitis	E. acervulina	E. maxima	E. necatrix	E. praecox	E. hagani	E. brunetti
Size in μ (Oocyst)	14.2-31.2X 9.5-24.8: av. 22.9X19.1	10.8-19.8X 9.2-17.3: av. 15.8X13.4	11.7-52.7X 9.2-21.1: av. 16.4X13	26.9-35.4X 17.7-29.8: av. 31.8X22.7	12.1-28.9X 10.1-18.8: av. 19.7X16.7	19.8-24.7X 15.7-19.8: av. 21.3X17.1	15.8-20.9X 14.3-19.5: av. 19.1X17.6	13.8-33.7X 12.1-26.2: av. 23.1X19.7
Shape	broad ovoid	Subspherical	Egg-shaped	Egg-shaped	Oblong ovoidal	Ovoidal	Broadly ovoid	Egg-shaped
Sporulation time	48 hr.* (18 hr.*)	48 b* (18 hr.*)	21 hr. (17 hr.)	48 hr. (30 hr.)	48 hr. (18 hr.)	48 hr.	36 hr. (16 hr.)	24-48 hr.
Prepatent period	6 days	4-5 days	4 days	5-6 days	6 days	4 days	6 days	5 days
Region of intestine chiefly parasitized	Schizonts and oocysts in ceca	Anterior small intestine	Anterior small intestine	Middle, anterior to posterior small intestine	Schizonts in small intestine, oocysts in ceca	Upper third of small intestine	Anterior half of small intestine	First generation schizonts throughout small intestine, later stages in ileum and posteriorly
Portion of parasites in tissue	Second generation schizonts subepithelial	Schizonts generally above and gametocytes generally below nuclei of epithelial cells	Above nuclei of epithelial cells	Schizonts above nuclei. Gametocytes deep in epithelium	Similar to E. tenella	Below nuclei of epithelial cells	?	Oocysts in all parts of cecal mucosa
Macroscopic lesions	Hemorrhagic ceca	None	Intestinal mucosa streaked transversely with whitish opacities composed of oocysts. Catarrhal exudate	Exudate or flecks of blood on mucosa; intestinal wall thick	Whitish opacities and hemorrhage and exudate in small intestine	None, except mucous cast of intestine	None	Catarrhal enteritis with blood-tinged exudate
Degree of pathogenicity	++++	+	++	++	++++	+	+	++++

*Sporulation times in patentheria are minimal at 29° C. reported by Edgar (1955a). See text for I. mavih. Edgar and Seibold (1964).

when pathology and other characteristics are disregarded, but in the case of the *E tenella* and *E. necatrix* there are certain stages that are indubitably peculiar to them. The second generation schizont of *E. tenella* commences its development in an epithelial cell of the cecal epithelium but soon the parasitized cell grows, becomes rounded, and migrates into the underlying connective tissue. These large schizonts, measuring up to 54_μ by 40_μ and subepithelial in position in the cecal wall, are peculiar to *E. tenella*. *E. necatrix* produces second-generation schizonts similar to those of *E. tenella*, often even larger, but they are located in the subepithelial tissues of the small intestine.

Transmission. The only accepted natural method of transmission has been ingestion of the viable sporulated oocysts by a susceptible host. However, several workers have succeeded experimentally in the transmission of infection by means of merozoites.

Krijgsman (1929b) states that Nöller succeeded in experimental transmission of coccidiosis to chicks through rectal injection of merozoite-containing material. Although Tyzzer (1929) had consistently failed to infect chicks with *Eimeria tenella* by cloacal injection of merozoites, Levine (1940c) accomplished this and also infected with merozoites injected directly into the small intestine through a catheter. He likewise succeeded in attempted merozoite infections of the crop and intestine of chicks with *Eimeria maxima, E. praecox, E. necatrix,* and *E. hagani.* These successes encouraged Levine to express the opinion that under favorable conditions merozoite infection occurs in the field.

Most infections have their incipiency in oocysts admitted into the digestive tract with food and drink or by fouling of the beak while scratching litter or preening In most poultry houses and runs where no special preventive measures are taken, there is ample opportunity for fecal material to lodge in wet drinking or feeding vessels and on damp litter or soil until sporulation has occurred. Under such con-

ditions the whole flock is likely to become infected sooner or later, with the probability that certain birds will acquire much more massive infections than others.

Dissemination of the oocysts is often more indirect. The hands, feet, and utensils of the attendants undoubtedly serve mechanically to convey infection from one building or pen to another. Flies have also been incriminated as mechanical vectors (Allen, 1932; Krijgsman, 1929a and b; Baker, 1933; and Wellman, 1954). Metelkin (1935) tested various species of wild and laboratory-bred muscoid flies and found them all capable of ingesting oocysts, which remained unaltered and viable in the insect gut up to 24 hours and in discharges until they dried. Delaplane and Stuart (1933) found that oocysts of avian coccidia in maggots were destroyed or eliminated in the process of development of larvae into adult flies. Less has been said about beetles, cockroaches, ants, and other invertebrates, but these are also suspected as mechanical vectors.

Birds and mammals visiting poultry runs probably carry oocysts about on their feet, but definite proof is lacking. There is, however, definite proof that oocysts, particularly the unsporulated, can pass through the intestines of certain animals and remain viable. Pérard (1933), for example, proved that dogs fed upon infected rabbit liver later egest the oocysts in such condition that they are capable of sporulating and infecting susceptible rabbits. Rats and mice may pass viable oocysts originating from other animals (Krijgsman, 1929b; Yakimoff and Iwanoff-Gobzem, 1931; Pérard, 1933; Bejšovec, 1960).

It is sometimes stated that both diseased and resistant recovered animals may act as contact carriers of coccidia. The statement is true, in a sense, but it is probably also true that a chick may be very severely stricken with bloody cecal coccidiosis on the fourth or fifth day of the infection and die before elimination of oocysts has commenced.

In *Eimeria tenella* infection, oocyst elimination commences late on the sixth day,

and sometimes becomes so intense on the seventh or eighth day that the cecal portions of the droppings consist of little else than oocysts and a small amount of fluid. Usually the numbers passed decline rapidly thereafter, until after a week or two they are difficult to find in the droppings at all. If, however, as often occurs in heavy infections, the cecal content commences to caseate on the sixth or seventh day, there may be practically no oocysts passed until days or weeks later when the cheesy core commences to liquefy.

Herrick, Ott, and Holmes (1936b) made a study of the length of time chickens may serve as carriers of *Eimeria tenella* during a single infection. Oocysts capable of sporulation and producing infections were found enmeshed in the cecal wall from 1½ to 7½ months following the infection date. In general, however, the longer the infection persisted the fewer were the number of oocysts found, and the higher was the percentage of them showing unmistakable signs of degeneration.

Warner (1933) found soil previously seeded with the oocysts of chicken coccidia infective for 197 days, but not 217 and 231 days. Delaplane and Stuart (1935), working in Rhode Island, demonstrated that the oocysts of avian coccidia survived in soil from experimental ranges for four to nine months following the removal of chickens. In soil from a wooded range the oocysts remained viable at 15 and 18 months. Farr and Wehr (1949) working in Maryland found that on shaded soil *E. acervulina*, *E. tenella*, and *E. maxima* oocysts remained infective for 602, 336, and 287 days respectively. (See also Koutz, 1950; and Kogan, 1959, 1960.)

A number of years ago the suggestion was frequently made that eggs become contaminated by excreta containing coccidia as they are being laid, and that young chicks can become infected by ingesting shell during the hatching process. Johnson (1923) early minimized the importance of this proposed method of transmission on the basis of the susceptibility of oocysts to drying and certain other fac-

tors. Tyzzer, Theiler, and Jones (1932) actually smeared eggs with fecal material and potassium dichromate solution containing *Eimeria necatrix* and *E. praecox* oocysts and incubated them. When the chicks began to pip the shell the fecal material was removed from the eggs and fed to susceptible chicks, but infection did not follow. Warner (1933) found that eggs dipped in suspensions of viable oocysts of poultry coccidia were not infective after 10 to 14 days of incubation at 40–70 per cent relative humidity and 38–40° C. temperature. Ellis (1938a), who made a more detailed study of *E. tenella*, found no viable sporulated oocysts on paper strips when they were kept at 45–70 per cent relative humidity and 100–104° F. for between 1 and 2 days, but at 91–93 per cent relative humidity and 100–104° F. they survived 3 and 4 days. Under conditions approximating those of normal egg incubation, sporulated oocysts did not live on the egg shell for more than a day or two.

The presence of a few oocysts in mash or in the feces of chickens does not necessarily mean that chicken coccidia are present. The junior author, while running a routine flotation on chicken feces, recovered along with a number of grain mites a few oocysts of a species of Barrouxia. Some of these oocysts were also found within the body of one of the grain mites. These oocysts, elliptical in shape and ranging in size between 22.5μ–26.2μ by 15.2μ–17μ, contained 10 to 15 spindle-shaped bivalve sporocysts and an oocystic residual body. Within each sporocyst was a single sporozoite.

Host-specificity. It is generally acknowledged that coccidia, particularly those of the common genus *Eimeria*, exhibit a marked degree of host-specificity. Evidence to support this will be found in many papers, some of the most pertinent of which are the following: Becker (1933), Corcuff (1928), Crooks (1934), Yakimoff and Ivanoff-Gobzem (1931), Yakimoff, Ivanoff-Gobzem, and Buewitsch (1932) ff and Gouseff (1933), Yakimoff, obzem, and Matschoulsky (19... (1962).

The experiments reported in these papers involved many Eimeria species of both mammalian and avian origin and many mammalian and avian hosts, but few indeed were successful intraspecific cross-infections, some of which should be discussed

Tyzzer and Jones (see Tyzzer, 1929) transferred *E. dispersa* from quail (bobwhite) to turkeys and occasionally to chickens, and *E. dispersa* from pheasants to quail, but second transfers in chickens and turkeys did not succeed. Hawkins (1952) transferred *E. dispersa* from turkeys to Hungarian partridges (*Perdix perdix*). Farr (1953) succeeded in transferring three species of Eimeria from Canada geese (*Branta canadensis*) to domestic geese (*Anser anser*). *E. meleagridis* of turkeys was passed to chickens and from chickens back to turkeys by Steward (1947) Gill (1954a) reported that three species of turkey coccidia, *E. meleagridis*, *E. meleagrimitis*, and *E. gallopavonis* were transmissible to chickens. It is to be noted that all of these successful attempts at cross-infection were between rather closely related groups.

The genus *Isospora* seems to show some laxity of host-specificity. One species, *Isospora lacazei*, has been reported from a number of unrelated passerine birds. Both Scholtyseck (1954, 1956) and Schwalbach (1959, 1960, 1961a), who have carried out experimental and taxonomic investigations on species of Isospora occurring in wild birds, suggested that *I. lacazei* may consist of two or more species. However, they have reported that, in many cases, a given species of Isospora has been found in many different species of birds.

There have been also certain claims of successful interspecific infections that are open to serious question in view of lack of subsequent confirmation. Henry's (1931) claims for successful passages of *E. tenella*, *E. acervulina*, and an *E. mitis*-like coccidium (all taken originally from two species of California quail) to baby chicks, have been severely questioned by Tyzzer, Theiler, and Jones (1932) on the basis of certain possible deficiencies in her procedure. Tyzzer (1929) had failed to infect twelve

chickens with *E. dispersa* from the quail, and Venard (1933) claims to have transmitted *E. tenella* of quail (bobwhite) origin to the chicken, although Patterson (1933) failed in infecting quail with *E. tenella*, *E. mitis*, *E. acervulina*, and *E. maxima* of chickens. Haase (1939) reported that he found *E. tenella* in quail (*Perdix perdix*). Thus host limitations of quail and chicken species require further investigation.

Many years ago wild birds, particularly the English sparrow, were blamed for the transmission of coccidiosis to fowls. Hadley (1910) discussed the English sparrow in terms that virtually condemned it as the source and disseminating agent in coccidiosis menacing the poultry-raising industry in all parts of the United States. Smith and Smillie (1917) and Johnson (1923), however, pointed out that only two spores appeared in the developing sparrow coccidium, while there were four in those from domesticated fowls.

Thus, the observed facts make it clear that, in general, coccidiosis in any particular species of bird or mammal is a problem more or less peculiar to it, and that "animal reservoirs" can usually be safely ruled out of consideration. It is not to be implied, however, that animals which frequent poultry runs, such as rats, mice, and sparrows, cannot act as passive disseminators.

Immunity. Flocks may almost imperceptibly develop more or less protective immunity to coccidiosis by repeatedly picking up small amounts of infective material. It has been emphasized time and again that the ideal type of environmental control of coccidiosis is one which permits this immunizing process to proceed, rather than to attempt to maintain the flock coccidia-free. In the latter event a severe outbreak would follow accidental introduction of the infection. Immunity resulting from previous infections is one reason older birds are more resistant to coccidiosis than younger ones. Immunization has also been produced by artificial inoculation with sporulated oocysts (Farr, 1943; Dickinson,

Babcock, and Osebold, 1951; Babcock and Dickinson, 1954; Pierce, Long, and Horton-Smith, 1962; Rose and Long, 1962). (Cf. Becker, 1934, pp. 11–13, 40; Jankiewicz and Scofield, 1934.) Herrick (1934) found that chicks displayed a natural resistance, and that certain chicks raised from parents that were particularly resistant to *E. tenella* were approximately 100 per cent more resistant than unselected chicks. (See also Champion, 1954; Edgar, King, and Johnson, 1951; and Rosenberg, Alicata, and Palafox, 1954.)

Various attempts have been made to attenuate the virulence of *Eimeria tenella* by treatment of the oocysts so as to effect immunity with a minimum of injury to the host. Jankiewicz and Scofield (1934) found that oocysts heated at 46° C. for 15 minutes before segmentation, and then fed to chickens after sporulation, conferred resistance to later inoculation with unheated oocysts and a minimum of injury to the host. Waxler (1941b) X-rayed the oocysts with 9,000r. Such oocysts, when fed to 35-day-old chicks, caused some drop in hemoglobin concentration but no deaths. The mild infection conferred almost as much resistance as a severe attack resulting from untreated oocysts. Uricchio (1953) fed oocysts kept frozen at −5° C. for 5 days to chicks 12 and 15 days old, with the result that they developed a marked protective immunity.

Until recently all attempts to determine the mechanism of immunity to coccidiosis have been unsuccessful. The evidence obtained by challenging previously infected birds with large doses of oocysts has suggested that the immunity might be restricted to the invaded tissue. In their experiments with ligated or isolated ceca Burns and Challey (1959) and Horton-Smith, Beattie, and Long (1961) showed that there was a more or less generalized response on the part of the chicken. Resistance to *E. tenella* acquired by infection of one cecum was transferred to the uninfected isolated cecum. Efforts to immunize birds or mammals with nonliving antigenic derivatives have been uniformly unsuccessful. (Cf. Pierce, Long, and Horton-Smith, 1963.) Becker and Zimmermann (1953) reported that chicks infected with *Eimeria tenella* and injected intravenously with an alcoholic extract of horse kidney discharged fewer oocysts during the infection than the untreated infected controls, though the reason remains obscure. Likewise, efforts to demonstrate antibodies in the circulating blood have generally been fruitless until McDermott and Stauber (1954) demonstrated agglutination of merozoites of *E. tenella* by means of sera from rabbits and roosters immunized with formalinized merozoite suspensions. They also demonstrated the agglutinins in the sera of experimentally infected chickens. Using the agar-gel diffusion technique, Pierce, Long, and Horton-Smith (1962) detected precipitating antibodies in the sera of chickens immunized with doses of *E. tenella* oocysts. Although these precipitins could usually be demonstrated during immunization, their presence was not essential for complete resistance. Rose and Long (1962) found that "serum precipitins were produced in infections with *E. maxima, E. acervulina, E. tenella* and *E. necatrix*. A first challenge of immune fowls with the immunizing species produced some increase in precipitation in agar whereas a second challenge had no such effect."

Although these investigations have shown that resistance is tied in with humoral mechanisms, the factors responsible for the immunity of chickens are yet to be elucidated.

Pathogenicity. Tyzzer, Theiler, and Jones (1932) state that in common poultry certain species of coccidia are practically innocuous, while others are capable of producing serious, destructive outbreaks of disease. *Eimeria mitis* and *E. praecox* are generally considered to be innocuous and *E. hagani* only slightly pathogenic.

The pre-eminently pathogenic species are *E. tenella*, which so attacks the cecal wall as to produce an acute, hemorrhagic type of disease, *E. necatrix*, which attacks the small intestine so as to produce either an acute initial attack resulting in early

death or a lingering illness characterized by progressive emaciation and general un-thriftiness, and *E. brunetti* which produces a necrotic enteritis in the lower half of the intestinal tract, causing more or less continuous losses in the flock. Of less importance as pathogens are *E. maxima* and *E. acervulina*. Heavy infections with these two species may cause weight losses and some deaths.

Since the life cycles of coccidia of the chicken are limited, and hence indefinite multiplication in the host is precluded, the size of the infective doses of sporulated oocysts bears a definite relation to pathogenicity. It has been found that very light doses may produce no clinically recognizable symptoms, and that up to a certain point morbidity and mortality increase in proportion to the size of the dose. (Cf. Tyzzer, 1929, Horton-Smith, 1947, 1949; Gardiner, 1955.)

Specific pathological peculiarities. E. meria tenella is the cause of so-called cecal or bloody coccidiosis of chicks (Figs. 37.3 and 37.4). Involvement of the ceca rather than of the small intestine is one of its characteristic features. Patillo (1959) and Burns and Challey (1959) reported that the sporozoites penetrate the surface epithelium of the cecal mucosa and migrate independently or are transported within macrophages through the lamina propria toward the muscularis mucosae. Along with the macrophages they enter the epithelium of the glands of Lieberkühn. The remainder of the life cycle is depicted on page 1067. The severity of this type of coccidiosis is attributable to the second generation schizont, which causes infected epithelial cells to increase tremendously in size and assume a migratory habit. Through pressure or otherwise there is produced sufficient degeneration of the blood vessels and surrounding tissues to result in bleeding into the ceca, and the copious bloody discharges from the ceca. The discharges usually commence to appear bloody sometime before the end of the fifth day after infection, but a certain wateriness of the droppings is sometimes noted much earlier.

The presence of thromboplastin in the cecal content of infected birds after the fourth day (i.e., after the hemorrhage occurs) has been demonstrated by the production of intravascular coagulation when such cecal content was injected intravenously into chickens (Bradford, Herrick, and Wolfe, 1947). The presence of the thromboplastin in the cecal content is explained by the disintegration of the cecal mucosa on the fifth day of the infection.

Levine and Herrick (1954) have shown by means of experiments in which gastrocnemius muscles of White Leghorns infected with *E. tenella* (fifth or sixth day) and of normal birds were electrically stimulated either through the sciatic nerve or directly, that the ability of that muscle to do work became greatly impaired during the infection and fatigued earlier and more severely. Since the same authors (1957) found that the muscles of infected White Leghorns did more work per contraction per gram than did those of Plymouth Rocks when stimulated directly, but not when stimulated through the nerve, the suggestion was made that the mechanism for the difference might be an effect of the end-plates or allied structures. A 50 per cent decrease in erythrocyte count and hematocrit value and a consequent decrease in blood volume entirely attributable to loss of erythrocytes were noted by Natt and Herrick (1955, 1956) on the fifth and sixth days of severe cecal coccidiosis. (See also Joyner and Davies, 1960; and Natt, 1959.) Bertke and Herrick (1954) noted petechial hemorrhages in the kidney parenchyma reaching maximum size by the end of the fourth day of the infection and accompanied by certain other alterations of the kidney structure. Schildt and Herrick (1955) noted that the motility of the crop and cecal pouches was seriously disturbed during *E. tenella* infection.

In their studies of bacterial populations in ceca of chickens infected with *E. tenella*, Johansson and Sarles (1948) reported that coliform organisms, primarily *Escherichia* species, did not decrease in numbers during the course of coccidiosis whereas lactic acid
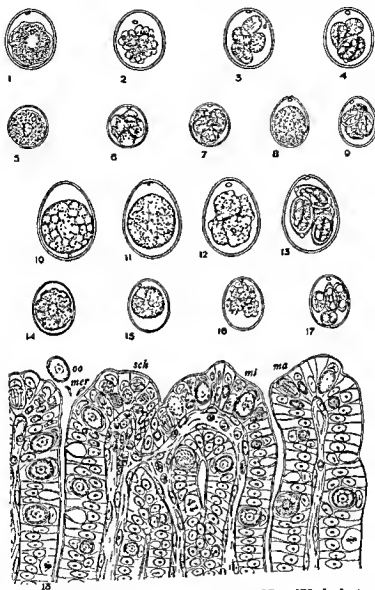
FIG. 37.3 — Five species of Eimeria found in chickens. 1–17, ×670. 1–4, stages in development of oocysts of E. tenella. 5–7, same for E. mitis. 8–9, same for E. acervulina. 10–13, same for E. maxima. 14–17, same for E. necatrix. 18, developmental stages in cecal epithelium from 7 to 9 days after infection. oo, oocyst. sch, third generation schizont. mer, third generation merozoite. mi, microgametocyte. ma, macrogametocyte. (All after Tyzzer, reproduced with permission of the Am. Jour. Hyg.)

producing bacteria almost disappeared. Conditions in the ceca were altered so as to favor proliferation of *Clostridium perfringens*. The authors suggested that *C. perfringens* and coliform organisms might be involved in the etiology of cecal coccidiosis. On the other hand Clark, Smith, and Dardas (1962) reported that, except for

delayed release of second generation merozoites, the course of *E. tenella* infection in bacteria-free chicks was the same as that of conventional chicks. The gross and microscopic lesions also appeared identical in gnotobiotic and conventional chicks.

Oocysts appear in the droppings commencing on the sixth day. Their dis-
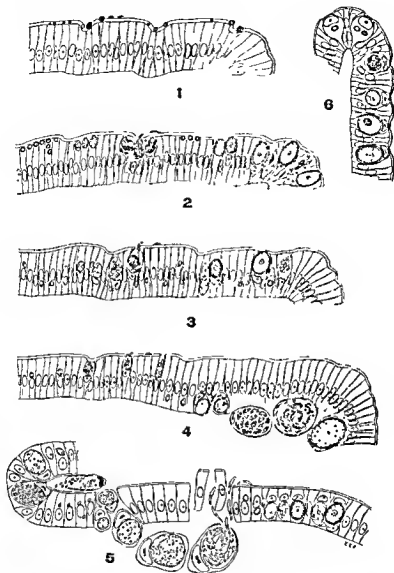
FIG. 37.4 – Diagram illustrating the situation of different species of coccidia in the intestine of fowls and the reaction of the parasitized epithelium. 1, Cryptosporidium parvum. 2, Eimeria acervulina. 3, E. mitis. 4, E. maxima. 5, E. tenella. 6, E. phasioni (in the pheasant). (All after Tyzzer, reproduced with permission of the Am. Jour. Hyg.)

charge continues more or less continuously over a considerable period. Herrick, Ott, and Holmes (1936b) found them in the droppings of chickens to 7½ months after the infection date. Their study showed that following infection the oocysts are enmeshed in the tissue of the ceca where they remain viable for at least 7½ months.

Eimeria necatrix attacks the small intestine, with the maximum involvement near the middle. According to Van Doorninck and Becker (1957), the sporozoites penetrate the epithelium of the villi and migrate through the lamina propria toward the muscularis mucosae. En route most of them are engulfed by macrophages which transport them into the epithelium of the fundi of the intestinal glands. The invaded

epithelial cells hypertrophy and migrate to the lumen of the gland fundus, meanwhile the parasites become first generation schizonts. The second generation schizonts are similar in form and behavior to those of *E. tenella*, and like them produce the injurious effects. On the fourth and fifth days aggregations of these schizonts appear as small whitish opacities. Later, punctate hemorrhages appear in the center of the whitish areas and may become so extensive as to obscure them altogether. As Tyzzer and collaborators state, "The unopened intestine thus presents a spotted appearance, the small whitish areas being intermingled with rounded, bright or dull red blotches of various sizes while transversely extending reddish streaks represent hemorrhages along the superficial vessels." There is profuse hemorrhage into the intestinal lumen. Joyner and Davies (1960) reported that the packed erythrocyte volume dropped 5 per cent within seven days after inoculation of oocysts.

Disease produced by *E. necatrix* may be of two types — acute or chronic. The former may result in the death of the bird 5 to 7 days after infection, while in the latter case the bird may linger on for a long time with a wasting illness. During the acute attack blood may be observed in the droppings.

In the case of *Eimeria necatrix* the first two generations of schizonts develop in the small intestine, the merozoites generated by the second generation schizonts migrate to the ceca where they invade the epithelium and develop, some into further generations of schizonts and some directly into oocysts. The ceca are little altered by the growth of the gametocytes and third generation schizonts. Blood drained from the small intestine may discolor the cecal contents.

Oocysts appear in the droppings on the seventh day after infection, and ordinarily require 2 days to sporulate. Tyzzer found that far more of them are produced in light infections than in heavy ones. An infected bird may discharge oocysts over a prolonged period. (See also Davies, 1956.)

Eimeria brunetti is the third of the definitely pathogenic species of the chicken.

Levine (1942c) indicated that the various stages of the parasite are distributed throughout the mucosa of the posterior half of the small intestine, rectum, ceca, and cloaca, and also the upper portion of the small intestine in heavy infections. In moderate infections there is a thickening of the gut wall, a pinkish or blood-tinged catarrhal exudate, and there also may be in the mucosa short, transverse red streaks, a millimeter or so in length, arranged in ladderlike fashion in long rows down the lower intestine and rectum. In severe infections there is an extensive coagulation necrosis and sloughing throughout the entire intestinal mucosa. Caseous cores may be found plugging the narrow portion of the ceca, but the dilated portions of the cecal wall are only moderately affected. According to Boles and Becker (1954) the first generation of schizonts develops in the epithelium of the entire small intestine and ceca. Later stages are usually concentrated in the lower digestive tract. Davies (1963) stated that *E. brunetti* "does not produce characteristic lesions in the intestine but infection is suggested by the presence of white caseous material in the lower intestine and rectum." (See also Pellérdy, 1961; Reid, Sharma, and Keener, 1961; Wiley, 1956; Zimmermann, 1957.)

Eimeria maxima, the fourth of the pathogenic species in the chicken, is far less lethal than the other three. Clinically, the recognition signs are dilation of the small intestine and thickening of the wall. The serous surface may show faint hemorrhages. The content is not bloody but takes the form of viscid mucus, grayish, brownish, or pinkish in color. In some instances the portion of the feces from the small intestine may show flecks of blood. Infections sometimes terminate fatally, but in general, large doses of oocysts produce temporary loss of weight, diarrhea, and a temporary reduction in egg production. (Cf. Johnson, 1931; Brackett and Bliznik, 1950; Long, 1959; Scholtyseck, 1959.)

Oocysts appear in the droppings on the sixth day after infection and continue for only a few days. After oocyst elimination ceases, the bird usually possesses a high de-

gree of immunity to reinfection. According to Long (1962) this resistance is short-lived. Chickens that showed marked resistance to reinfection 4 weeks after their first infection were highly susceptible when reinoculated 10 weeks later. The oocysts of this species are quite characteristic, being the largest of all occurring in chickens (about 29.3μ by 22.6μ on an average) and having a slightly roughened wall.

Eimeria acervulina is, fortunately, not a severe pathogen, though it is perhaps the commonest of all the poultry coccidia. It is characterized clinically by numerous gray or whitish patches in the upper half of the small intestine, visible through the serous surface. These patches are caused by forming oocysts.

That the species is not very pathogenic is quite likely a fair statement of the situation. Heavy doses, however, result in considerable morbidity and mortality (Gill, 1954b; Moynihan, 1950; Gill and Lall, 1961; Horton-Smith and Long, 1959a and b; Morehouse and McGuire, 1956).

In the northwestern part of the United States this species has a serious effect on pullets three or four weeks after housing. (See Peterson, 1949; Peterson and Munro, 1949; Dickinson, 1939, 1941, 1949.) The symptoms noted were shriveled combs, weight loss, and cessation of egg production. Flock culling at this time results in loss of about a fourth of the birds. Egg production is renewed after the disease has run its course. The affected birds reveal at necropsy the lesions of *E. acervulina* throughout the upper portion of the small intestine and great numbers of oocysts within the gut lumen. Thus it appears that under certain conditions *E. acervulina* is not the almost innocuous parasite that it appears to be under usual conditions.

Eimeria mitis grows in the small intestine throughout its entire length, but is most concentrated in the upper half. It is definitely not a serious pathogen. The oocysts are small, with a tendency to be spherical, and usually are not abundant in the droppings. Joyner (1958) reported that large doses of oocysts caused (1) weight retardation and limited mortality in very young chicks, and (2) a reduction in weight gain in chicks 17 to 26 days old. He found no evidence of hemorrhage and no gross lesions in any infected chicken.

Eimeria praecox develops in the upper third of the small intestine. Tyzzer, Theiler, and Jones (1932) stated that this species "elicits no appreciable inflammatory reaction even in heavy infections, so it may be regarded as practically innocuous as far as direct injury to the tissue is concerned." Johnson (1931) found that feeding of large doses of oocysts to mature, highly susceptible White Leghorn chickens was followed by a slight average decrease in egg production. (See also Levine, 1945.)

Eimeria hagani occurs chiefly in the upper half of the small intestine. Levine (1938) reported that infection produces round hemorrhage spots the size of a pinhead and a severe catarrhal enteritis in the duodenum and upper half of the remainder of the small intestine, and comparatively few such lesions in the lower half. Later Levine (1942a, 1945) concluded that this species is relatively nonpathogenic and that it does not produce any characteristic lesions by which it can be recognized.

Eimeria mivati was established as a new species by Edgar and Seibold (1964). It is said to be moderately pathogenic, producing congestion, petechiae, and whitish opacities principally in the upper third of the small intestine with some spread into the lower small intestine, ceca, and rectum. The schizonts and gametocytes are located above the host cell nuclei in the upper intestine and usually below the nuclei in the lower tract. The oocysts (11.1μ–19.9μ by 10.5μ–16.2μ) are ellipsoid to broadly ovoid. The minimum sporulation time is 12 hours. The prepatent period is 93 hours and the infection lasts from 6 to 12 days. Ray (1945) has described *Wenyonella gallinae* from 4- to 6-week-old chickens in India. Sporulated oocysts of the genus *Wenyonella*, like *Eimeria*, contain four spores, and each of the latter, like *Isospora*, contains four sporozoites. The oval oocysts presented a punctate surface,

rough in optical section, and measured $29.5\mu–33.5\mu$ by $19.8\mu–22.8\mu$. The characteristically flask-shaped sporocysts measured 18.8μ by 8.0μ. At $28°$ C. in 2.5 per cent potassium dichromate solution, sporulation required 4 to 6 days. The infection was characterized by (1) blackish-green, semisolid excreta and intestinal content containing numerous oocysts and (2) pinpoint hemorrhages in the mucosa and thickening and congestion of the terminal part of the intestine. Gill (1954c) reported that 1.7 per cent of fecal samples collected from various parts of India contained oocysts of this species.

Scholtyseck (1954) found, in feces of three hens, unsporulated oocysts whose zygote almost filled the space enclosed by the wall. The oocysts, oval in shape and without a micropyle, ranged in approximate size between $19\mu–27\mu$ by $15.5\mu–23\mu$. When sporulated they possessed two pyriform sporocysts each containing four sporozoites. No transmissions were reported. The species was named *Isospora gallinae*. The validity of this species is questionable.

Tyzzer (1929) found a species of *Cryptosporidium* growing in the cuticular layer of the cecal epithelium in a few chickens. He tentatively identified the parasite as *C. parvum* Tyzzer, 1912, a species originally described from the house mouse. Levine (1961), doubting the accuracy of Tyzzer's identification, gave the chicken form "a name of its own," *C. tyzzeri*. According to Levine the oocyst measures $4\mu–5\mu$ by 3μ and contains four free sporozoites and an oocystic residual body.

Histopathology. We are indebted largely to Tyzzer (1929); Tyzzer, Theiler, and Jones (1932); and Mayhew (1937) for knowledge concerning the histopathology in coccidiosis of the chicken. *E. tenella* is probably by far the most important of the severe pathogens.

According to Pattillo (1959), penetration by groups of *E. tenella* sporozoites produced damaging passageways or "penetration tubes" in the epithelium of villi tips.

After transportation through the tunica propria and into the base of an epithelial cell of a cecal gland, the sporozoite rounds up and grows into a large schizont. The schizont develops about 900 merozoites and moves out through the distal end of the cell into the lumen of the gland, pushing the host cell nucleus before it. The merozoites escape from the schizont, penetrate adjacent epithelial cells and start to grow, causing the host cells to round up and assume wandering habits. The host cells migrate into the mucosa and submucosa where they and their parasites increase in size to such an extent that by virtue of volume and numbers the cecal wall becomes congested, blood vessels become disrupted, and leakage of blood ensues. Thus is explained the hemorrhage that sometimes commences late on the fourth day of a heavy attack and persists through the sixth day. According to Tyzzer, the bird may literally bleed to death.

Mayhew (1937) found that in light attacks severe damage to the tissue is but local and any destroyed epithelial or underlying tissues are regenerated. If there has been severe bleeding, a core will form in the lumen. In heavy attacks, on the other hand, a considerable area of the mucosa and submucosa may become congested, and the developing parasites may cause such disintegration of the tissue elements that the layers of the mucosa and submucosa lose their identity. There is such profuse discharge of blood cells, lymph, parasites, and tissue cells into the lumen of the cecum as to form a clot or core fitting the form of the ceca. The core to the cecal wall is adherent at first, but in a few days the surface liquefies so as to free it. It may be passed with the feces in the course of time, though sometimes the cores are retained. Allen (1934) and Mayhew (1937) have discussed the latter condition. As the birds recover from the severe form of the disease, the epithelium in the glands and the tunica propria are restored, but in the most severely afflicted birds the surface epithelium between the glands is not renewed. (See also Gill and Ray, 1957; Greven, 1953; Inagaki and Tsubokura, 1954; and Scholtyseck, 1953.)

Histochemical studies have been made on the life cycle stages and lesions of several of the chicken species of Eimeria. For a detailed discussion of cytochemical work on both chicken and rabbit coccidia see Horton-Smith and Long (1963). (See also Gill and Ray, 1954a, b; Long and Rootes, 1959; Monné and Honig, 1954; Pattillo and Becker, 1955; Pattillo, 1957; Ray and Gill, 1954, 1955; Tsunoda and Itakawa, 1955; and Wilson and Fairbairn, 1961.)

Seasonal incidence. Under ordinary farm conditions, most outbreaks of coccidiosis occur during the months of May, June, July, and August. This is clearly shown by Durant and McDougle (1939) in a graph of 838 necropsied chickens in Missouri. Krassner (1963) observed that yields of E. acervulina oocysts from birds of comparable ages were higher in winter than in summer. In broiler raising, however, chicks are reared throughout the year, and outbreaks may occur at any time.

Age factor. Cecal coccidiosis occurs principally in young chicks, but seldom in those less than 10 or 11 days old. Edgar (1955e, 1962) stated that when chickens were inoculated with numbers of E. tenella oocysts in proportion to body weight, there was significantly less mortality in those inoculated at three days of age than among those inoculated when older. The greatest mortality was among chicks inoculated at four weeks of age. Nonexposed chickens were highly susceptible at one year of age (See also Gordeuk, Bressler, and Glantz, 1951.) Many of the worst outbreaks occur at the age of six to eight weeks. Herrick, Ott, and Holmes (1936a) in their study of experimental infections in chickens of different ages found that the heaviest mortality (72 per cent) and the greatest decrease in erythrocytes (60 per cent decrease) occurred in chicks one month old. Mortality and red cell decrease were also heavy in one-half-month- and two-month-old chicks, whereas in older birds (three to ten months) mortality was low or lacking, though the drop in red cell counts ranged from 29 per cent to 46.8 per cent. Gardiner

(1955), who employed dosages of 50,000, 100,000, and 200,000 sporulated oocysts, infected young chickens in age groups of 1, 2, 3, 4, 5, and 6 weeks. Those in the 4-week group were, in agreement with the findings of Herrick, Ott, and Holmes, die most severely affected, and those in die 2-week group the least. Brackett and Bliznick (1952b) and Davies (1956) found that with equal doses of oocysts, E. necatrix was more pathogenic for young chickens than for older ones. However, Brackett and Bliznick (1952b) noted that when the size of the dose of oocysts was in proportion to the body weight, the older birds might be more severely affected than younger birds. Long (1959) working with E. maxima and Krassner (1963) with E. acervulina concluded that on the basis of oocyst output older birds were more susceptible to these species than younger ones. Levine (1940a), in a study of subclinical coccidial infection in pullets at least eight months old, reported that these older birds were serving as abundant sources of E. mitis, E. acervulina, E. praecox, E. maxima, E. necatrix, and E. tenella, although only 8 per cent showed gross lesions of coccidiosis.

Effect on development and egg production. Mayhew (1932a, b; 1934b) has found that birds inoculated during the seventh or thirteenth and fourteenth weeks were definitely handicapped in that they do not regain the weight lost during an attack in the following three months; i.e., as compared with the uninfected controls. In a later study he showed that hens developed from chicks inoculated at the age of six to eight weeks laid 19.25 per cent fewer eggs than the controls, and did not attain normal weight (as determined by controls) until five or six months after the attack. Johnson (1931) found that inoculation of mature S.C. White Leghorns with large doses of either E. acervulina, E. maxima, or E. tenella oocysts resulted in complete temporary cessation of egg production. Dickinson (1941) reported that pullets receiving massive doses of E. acervulina oocysts lost weight temporarily and ceased laying for 7 to 12 days. Within a

month they had returned to normal. According to Berg, Hamilton, and Bearse (1951) a small dose of *E. maxima* oocysts had a similar effect on the egg production of White Leghorn pullets. (See also Bressler and Gordeuk, 1951; Edgar, 1960.)

Physiological effects. Severe cecal coccidiosis, with loss of blood, causes a rise in blood sugar during the fifth, sixth, and seventh days of the infection. Artificial bleeding produces the same effect, while starvation does not (Pratt, 1940). Pratt (1941) later found that on the sixth day of the infection the glycogen content of the bird's muscle was less than half of that in normal birds starved 19 hours, while the liver glycogen was slightly higher, though more variable than normal. Daugherty and Herrick (1952) suggested that the increased blood sugar level in cecal coccidiosis is due not so much to bleeding as to possible interference with carbohydrate metabolism; they were able to demonstrate, in support of the hypothesis, interference with phosphorylative carbohydrate dissimilation by homogenates of tissues by an unidentified material present in the cecum of infected fowls. Also, they reported that the R.Q. (respiratory quotient) values of muscle tissue from infected fowls were lower than the normal on the fifth and sixth days.

Challey (1960) observed that both acute cecal coccidiosis and artificial hemorrhage caused a marked increase in ascorbic acid in the adrenals of chickens. He suggested that the adrenal ascorbic acid elevation might be due to blood loss alone. Challey (1962) reported that adrenal corticosterone concentrations were also elevated in chicks during the early hemorrhagic phase of cecal coccidiosis.

Waxler (1911a) has demonstrated that feeding concentrated physiological salt solution to birds during the hemorrhagic phase of the disease effects a lesser rise in blood sugar than when no salt is fed. In addition, the mortality was three times greater in the untreated birds than in the salt-fed. However, he was not able to positively attribute the beneficial effects of salt

feeding to the lower rise in blood sugar.

Prevention. The ideal control of poultry coccidioses would seem to consist simply of preventing the ingestion of viable sporulated oocysts by susceptible hosts. The difficulties lie in perfecting methods of achieving this desideratum. One difficulty is that the oocysts of most of the species occurring in chickens will survive in soil for as long as a year and more.

While extremely low temperatures, such as −12° C. for seven days (Edgar, 1954), will kill the oocysts of *Eimeria tenella*, Horton-Smith (1957b) thinks it is the moderately low winter temperatures, preventing the fresh oocysts from sporulating or reducing the rate at which sporulation proceeds, that are most important in the epidemiology of coccidiosis. Other environmental factors that unfavorably affect the survival of oocysts are dryness, direct sunlight, heat, lack of oxygen, and bacterial and fungal action.

The findings of Andrews and Tsuchiya (1931) showed that on a poultry farm the greatest concentrations of oocysts are built up in places where the birds spend the most of their time. Devices to prevent contact of the birds with their droppings in such areas should, and in actual practice do, reduce losses from coccidiosis, as Van Es and Olney (1940) indicated.

The frequent removal and replacement of the litter is recommended also, but despite all these precautions, the problem often remains unsolved.

The thorough cleansing of the floor and equipment in a brooder house between each group of chicks brooded is helpful. After the thorough removal of filth, floor and utensils should be thoroughly scrubbed with hot lye water. Sawyer and Hamilton (1935) recommended 1 pound of lye to 20 gallons of water. Afterwards floor and equipment should be thoroughly dried for, as Pérard (1925) has demonstrated, drying is probably one of the most potent natural forces in the destruction of oocysts.

Of all the fumigation methods that

have been proposed, ammonia fumigation seems the most practicable. Horton-Smith, Taylor, and Turtle (1940) obtained complete killing of the oocysts of *E. tenella* with a 1.0 per cent solution of 0.83 per cent ammonia in 24 hours, with a 5.0 per cent solution in 2 hours, and with a 10 per cent solution in 45 minutes.

It is important that the attendant takes care that he does not track fecal material from one house or pen to another.

The form of range management advocated is the three-year rotation plan, wherein the range is used for chicks but one year and planted to a cereal crop and grass the next two years. Under this plan the brooder houses are of the movable type. Each house is thoroughly cleaned before it is transported into the clean range.

The "deep litter method" of controlling poultry coccidiosis was tested by Boughton (1939). It consists of the use of a deep layer of sawdust or shavings, stirred daily, over the entire period of brooding, i.e., ten or twelve weeks. The litter is renewed just before the advent of the new brood of chicks. Boughton's preliminary study did show that such litter, when dry, does reduce the potential number of sporulated oocysts through drying.

Improvements in the deep and built-up litter methods consist of better insulation and ventilation of the poultry house, which favor dryness of the litter, and the use of hydrated lime in the litter. Kennard and Chamberlin (1947) have adopted the following procedure for combining the built-up litter and hydrated lime practices in the brooder houses: At intervals of 2 to 4 weeks hydrated lime is scattered over the litter at the rate of 10 to 15 lb. per 100 square feet of floor space, and carefully mixed with the litter at time of distribution in order to avoid caustic effects on the feet of the birds. The litter is stirred and redistributed every 2 or 3 days during the first 8 weeks and daily after that. Litter and lime may be added at any time as needed, but lime is seldom needed after the first 4 or 5 weeks. Such litter will remain dry and fresh for 8 to 16 weeks. Particular care should be taken to redistribute the litter about the water fountains and feed boxes. The Ohio Station had no coccidiosis in a brooder house in a season during which five successive broods, totaling 10,000 chicks, occupied the structure. Koutz (1918) doubted the value of liming deep litter, though his investigation proved the value of deep litter, properly managed, in helping the birds to build up protective resistance to *E. tenella* with minimal losses from the disease. In this and later work Koutz (1952a, b) showed that many oocysts of *E. tenella* and other coccidia, as well as ova of parasitic nematodes, remain alive, so that continuously used litter develops a high and dangerous concentration of them. For these reasons, and also because of increased amounts of dust and ammonia fumes, there are certain hazards to the life and health of successive groups of birds reared under the so-called continuous, built-up, deep litter method. Horton-Smith (1954) has pointed out similar dangers inherent in built-up litter when its depth is not maintained, but has noted that proper management of deep litter would reduce the number of viable oocysts, presumably largely through the toxic action of the ammonia produced. (See also Davies and Joyner, 1955; Long and Binstead, 1959.)

Treatment. The advent of the sulfonamides marked a new era in suppressive and therapeutic drug treatment of coccidiosis. Levine (1939) made the interesting discovery that sulfanilamide suppressed the normal development of *E. mitis*, *E. hagani*, *E. pmecox*, *E. acervulina*, and *E. maxima*, so that oocysts did not appear at the anticipated date, though a reduced number of the terminal stages appeared a few days after treatment was discontinued. There was no effect of this drug upon *E. tenella* and *E. necatrix*, the two most pathogenic species which, incidentally, produce schi-

zonts deep in the submucosa. Sulfapyri-
dine's action almost paralleled that of sul-
fanilamide (Levine, 1940b).

Sulfaguanidine, however, when mixed
with the ration at the ½ per cent level,
prevented discharge of oocysts from chick-
ens inoculated with *E. praecox, E. mitis, E.
maxima,* and *E. hagani,* according to Le-
vine (1941a). At the 1 per cent level it
markedly reduced the severity of symp-
toms and lesions due to *E. tenella,* while
a 1½ per cent concentration was effective
against *E. necatrix.* Farr and Allen (1942),
Horton-Smith (1942), and Allen and Farr
(1943) have attested to the prophylactic
value of sulfaguanidine against cecal coc-
cidiosis in mash containing 1 or 2 per cent
of the drug, providing the treatment is in-
stituted several days before ingestion of
infective oocysts and continued for more
than a week thereafter. It would seem
from the work of Waletzky and Hughes
(1946) that 0.75 per cent might be the mini-
mal requirement for marked prophylactic
value.

Horton-Smith and Taylor (1942, 1943,
1945) obtained beneficial results with
sulfamerazine (=sulfamethazine) and
sulfadiazine in the food or drinking water
after establishment of the cecal infection
in chicks through induced epizootics.
Treatment reduced mortality by 50 to 73
per cent of that in the controls. Hawkins
(1943) confirmed this work in general,
and noted inhibition of the cecal coccid-
iosis by substituting a saturated solution
of sulfamethazine for drinking water 96
hours after infection. Hawkins and Kline
(1945) found that 0.4 to 1.0 per cent in
the feed, started 4 days after infection,
gave more constant results than solutions
in drinking water. Farr and Wehr (1947)
and Wehr and Farr (1947) attributed the
beneficial effects of sulfamethazine in *E.
tenella* infection to the susceptibility of
the second generation schizonts to the
toxic effects of the drug.

Swales (1914, 1946a) confirmed Horton-
Smith's work on sulfamethazine (=sulfa-
methazine), and showed that related

compounds, sulfamerazine and its sodium
salt, were highly coccidiostatic and would
check the disease when treatment was
started at the first sign of bloody drop-
pings in the flock. The levels found satis-
factory were 2 gm. of sulfamerazine per
pound of dry mash or 2 gm. of its soluble
sodium salt per liter of drinking water.
There was no advantage in continuing the
treatment for more than 3 days. (See also
Hawkins and Rausch, 1946; Gardiner,
1957a.) Horton-Smith and Boyland
(1946) have effectively treated *E. tenella*
infections with 0.2 per cent sodium sul-
famezathine or 0.1 per cent sodium sulfa-
pyrazine in the drinking water. Asplin,
Boyland, and Horton-Smith (1946) have
issued the warning, however, that because
of the dangers involved in long-continued
administration, sulfamezathine treatment
should not exceed one week.

Horton-Smith (1957a) showed the rela-
tive effects of sulfanilamide, sulfapyridine,
sulfathiazol, sulfaguanidine, sulfadiazine,
sulfamerazine, sulfamezathine, and sulfa-
pyrazine on the second generation schizonts
of *Eimeria tenella* and on mortality when
administered 48 hours before infection and
48 hours afterwards.

The action of sulfonamides in coccidial
infection is antagonized by para-amino-
benzoic acid (cf. Horton-Smith and Boy-
land, 1946; Waletzky and Hughes, 1946),
a phenomenon which might indicate that
the coccidiostatic effect of the sulfon-
amides is upon the PAB-folic acid meta-
bolic sequence. Continuing the work
along this line in search of more potent
inhibitors, Lux (1954) not only found
certain diaminopyrimidines and dihydro-
triazenes that played this role effectively,
but he discovered synergism between 2,4-
diamino compounds and certain sulfon-
amides. Even sulfanilamide, which at 0.5
per cent level in the ration permitted 89
per cent mortality with the dosage of *E.
tenella* employed, when fed at the 0.025
per cent level together with 2-4-diamino-5-
p-chlorophenyl-6-ethylpyrimidine at the
0.005 per cent level, prevented mortality

altogether. The potentiation of otherwise ineffective doses of sulfadimidine, sulfaquinoxaline, and sulfaguanidine by pyrimethamine has been reported by Joyner and Kendall (1955) and Kendall and Joyner (1956). (See also Arundel, 1959; and Joyner, 1960.) Another seeming synergism is that between aureomycin and sulamethazine (Gardiner, 1957b, 1959). Levels of these chemicals, which by themselves were ineffective in preventing mortality and promoting growth under conditions of exposure to cecal coccidiosis, when combined in the mash reduced the pathological changes identified with cecal coccidiosis.

Other forms of drug prophylaxis or therapy in cecal coccidiosis are: (1) certain halogenated arsenic acids and their sodium salts at the proper concentrations in feed or water (Morehouse, 1946; Morehouse and Mayfield, 1946; and Goble, 1949;) (2) nitrofurans such as 5-nitro-2-furaldehyde semicarbazone ("nitrofurazone") in the mash (Harwood and Stunz, 1949a, 1949b, 1950; Horton-Smith and Long, 1952; Gardiner and Farr, 1954); furazolidone (about .006 per cent in the feed) (Harwood and Stunz, 1954; Wolfgang, et al., 1957; Harwood, et al., 1957); and Bifuran—a mixture of nitrofurazone and furazolidone (Horton-Smith and Long, 1959a and b; and McLoughlin and Chester, 1959); (3) m,m'-dinitrodiphenyl disulfide ("nitrophenide," "Megasul") in the mash (Waletzky and Hughes, 1946; Waletzky et al., 1949; Brackett and Bliznick, 1949; Swales, 1950; Dickinson et al., 1951; Gardiner et al., 1952); (4) sulfaquinoxaline in an all-mash ration (Delaplane et al., 1947; Delaplane and Higgins, 1948; Grumbles and Delaplane, 1948; Grumbles et al., 1948; Grumbles et al., 1949; Peterson, 1948; Junghern and Winn, 1949; Brackett and Bliznick, 1949); (5) bisphenols ("K6606," "K6605," "K1409," etc.) in the feed (Johnson et al., 1949; Hawkins and Dunlap, 1949); (6) nicarbazin (0.0125 per cent) in the ration (Guckler et al., 1955; Guckler et al., 1956; Guckler and Malanga, 1956; Rubin et al., 1956; McLoughlin et al., 1957; (7) Trithiadol—a combination of bithionol and methiotriazamine (Mc-

Loughlin and Chester, 1959; McLoughlin et al., 1960; Stuart et al., 1963); (8) Glycarbylamide (Horton-Smith and Long, 1959a and b; McLoughlin et al., 1960); (9) Amprolium (Cuckler et al., 1960; McLoughlin and Gardiner, 1962a and b); (10) Zoalene (Peterson, 1960); (11) Unistat (Morehouse and McGuire; 1959); (12) vitamin K (Baldwin et al., 1911; Couch, 1951; Harms and Tugwell, 1956; Otto et al., 1958).

Nitrophenide and sulfaquinoxaline are claimed to be effective against both Eimeria tenella and E. necatrix (Waletzky, et al., 1949). Peterson and Munro (1949) have found sulfaquinoxaline to be of value in preventing coccidiosis with E. acervulina in recently housed pullets, and Dickinson (1949) showed it to be an effective coccidiostat against artificial infection with E. acervulina in laying pullets without affecting body weight or egg production at the levels fed. Factors in the evaluation of coccidiostats in poultry have been discussed by Cuckler et al., (1957), with a bias toward nicarbazin.

Tyzzer (1929), Johnson (1932), and others of the early workers have pointed out the possible value of small doses of sporulated oocysts either fortuitously picked up or intentionally administered in the mash as a means of establishing in young chickens a considerable resistance against infection resulting from heavier doses of the infective microorganisms acquired later in life.

With the advent of drug prophylaxis there has become evident the desire of experimenters to show that various drug coccidiostats, while preventing serious disease, do not interfere with the natural development of immunity to coccidial infections acquired while the birds are under treatment. Ripsom and Herrick (1945) showed that sulfadiazine in the mash at the 1.0 per cent level, for 24 hours commencing the sixth day of an experimental E. tenella infection, protected the test birds and did not interfere with the development of immunity. The potential value of sulfaguanidine for establishing

resistance to infective oocysts of *Eimeria tenella* was demonstrated by Allen and Farr (1943) and Seeger and Tomhave (1946). Swales (1946a and b) found that feeding sulfamerazine in the mash early in the infection did not interfere with the development of immunity to *Eimeria tenella*. (For the effect of other sulfonamides see Horton-Smith, 1948.) Dickinson *et al.* (1951) reported that chicks were inoculated at 15 days of age with a "mild clinical dosage" of *Eimeria tenella, E. necatrix, E. maxima, E. praecox,* and *E. acervulina.* Commencing 48 hours after the coccidial inoculation, they were fed, for 14 days, mash containing 0.025 per cent sulfaquinoxaline and were almost as well protected against heavier challenging doses administered at the age of 57 days as were those in the immunized untreated group. Another group, treated the same except that it received nitrophenide in the mash at the 0.05 per cent level, suffered from the challenging doses of infected oocysts almost as much as the nonimmunized, untreated controls. That nitrofurazone, fed to chicks at levels for efficient coccidiostatic action, did not interfere with the development of resistance to *E. tenella* is suggested by the works of Horton-Smith and Long (1952) and Gardiner and Farr (1954). The latter actually felt that when this drug was fed at levels to provide only partial protection, the development of resistance was actually enhanced. Cuckler and Malanga (1956) found that nicarbazin did not adversely affect the immunizing process, although it significantly reduced the pathological effects of avian coccidiosis as well as the numbers of oocysts eliminated. (See also McLoughlin *et al.*, 1957.) Studies on the tissue phases of *E. tenella* have shown that drugs which effectively reduce the pathological effects of the organism while not affecting the immunizing process act chiefly on the second generation schizonts. (See also Horton-Smith, 1957a.)

Success in developing anticoccidial drugs has been attended with the revelation of toxic properties. All of them are toxic

when fed in the mash or drinking water at levels in excess of the manufacturers' recommendations. Lack of knowledge of this fact and of the proper equipment for uniform mixing render it inadvisable for other than the feed manufacturer to add the drug supplements to the ration. Even when fed at or near the recommended levels, unfavorable effects may become manifest. Considerable attention has been paid to blemished or mottled egg yolks, reduced egg size, egg production, and bleached shells when nicarbazin is fed to laying hens (Ott, *et al.*, 1955; Sherwood *et al.*, 1956; Polin *et al.*, 1956 and 1957; McLary, 1955; Newberne and Buck, 1957; Weiss, 1957; Baker *et al.*, 1956 and 1957). Other toxicological studies of note have been made on the following anticoccidial drugs: sulfaquinoxaline, Cuckler and Ott (1955), Newberne and Buck (1956); Megasul, Newberne and McDougle (1956); nitrofurazone, Newberne and McEuen (1957), Peterson and Hymas (1950), Francis and Shaffner (1956); furazolidone, Berg *et al.* (1956), Francis and Shaffner (1956). It has been pointed out that dependence on drugs in raising poultry should not be substituted for sanitary practices, and that medication of any sort should accompany efficient management (see Horton-Smith, 1957a). Anticoccidial drugs have their proper place in poultry management, especially so in preventing losses from cecal coccidiosis in the broiler industry.

The development of drug resistance by coccidia seems first to have been investigated by Harwood and Stunz (1953), who found none in infections with three cultures of *Eimeria tenella* from strains that had long been exposed to drug treatment with either nitrofurazone or sulfaquinoxaline. On the other hand, Cuckler and Malanga (1955) noted loss of sensitivity to a therapeutically ineffective concentration of sulfaquinoxaline by one strain of *E. acervulina* after 10 serial passages and by two strains of *E. tenella* after 5 to 10 passages; but one strain of *E. tenella* did not become less sensitive to suboptimal

doses of nitrophenide, nitrofurazone, or nicarbazin for 15 serial passages. In addition, their studies on 40 field strains of allegedly resistant coccidia revealed 43 per cent were resistant to nitrophenide, 45 per cent to sulfaquinoxaline, and 57 per cent to nitrofurazone. It was not determined whether drug resistance in coccidia is attributable to selective drug action among different strains of the microorganism possessing different degrees of innate susceptibility (See also McLoughlin and Gardiner, 1961, 1962a and b; Gardiner and McLoughlin, 1963.)

Swales's (1947a) "System C" for controlling coccidiosis provides that battery-raised susceptible 3- or 4-week-old chicks be placed in an "immunizing pen" whose floor has previously been contaminated with the droppings of heavily infected chicks, and be given preventive treatment of such drugs as sulfamerazine, sodium sulfamerazine, sulfamezathine, or sodium sulfamezathine for 6 to 10 days. The birds are then placed in clean dry pens for development to broilers or layers.

Edgar (1955b, 1956, 1962) has developed a vaccine for intestinal and cecal coccidiosis which is claimed to achieve regulated immunization of chicks on a commercial scale. Under his plan, 3-day-old chicks are starved for about three hours and then fed a feed freshly mixed with a commercially prepared culture of sporulated oocysts of *E. tenella* and three to five other species of chicken coccidia. The chicks develop mild infections and seed the litter with oocysts which undergo sporulation and in turn become infective. These infective oocysts are picked up by the birds on the newly contaminated litter, and reinfections result. To prevent serious disease, a low level of a coccidiostat is administered until the birds are 5 to 6 weeks old. The chicks are supposed to have acquired enough partial immunity during this period to withstand further exposures. Levine (1961) stated that although this system has often worked well, there have been too many failures to justify recommending its general use.

REFERENCES

Allen, E. A.: 1932. The influence of diet on the development of experimental coccidiosis in chickens kept under sanitary conditions. Am. Jour. Hyg. 15:163.
———.: 1933. The pathogenicity of *Eimeria mitis* Tyzzer, 1929, to 3-month-old chickens. Jour. Parasit. 20:73.
———.: 1934. A case of prolonged cecal coccidiosis. Proc. Helminth. Soc. Wash. 1:66.
Allen, R. W., and Farr, M. M.: 1943. Sulfaguanidine as a prophylactic during the period of acquirement of resistance by chickens to cecal coccidiosis. Am. Jour. Vet. Res. 4:50.
Andrews, J., and Tsuchiya, H.: 1931. The distribution of coccidial oocysts on a poultry farm in Maryland. Poultry Sci. 10:320.
Arundel, J. H.: 1959. The efficiency and toxicity of pyrimethamine in the control of caecal coccidiosis of chickens. Austral. Vet. Jour. 35:7.
Asplin, F. D., Boyland, E., and Horton Smith, C.: 1946. Treatment of caecal coccidiosis of chickens by sulphonamides. Biochem. Jour. 40 li.
Babcock, W. E., and Dickinson, E. M.: 1954. Coccidial immunity studies in chickens. 2. the dosage of *Eimeria tenella* and time required for immunity to develop in chickens. Poultry Sci. 33:596.
Baker, A. D.: 1933. Some studies of the dipterous fauna of the poultry yard in Quebec in relation to parasitic troubles. Poultry Sci. 12:42.
Baker, R. C., Hill, F. W., van Tienhoven, A., and Bruckner, J. H.: 1956. Effect of nicarbazin on egg quality. Poultry Sci. 35:1132.
——— Hill, F. W., van Tienhoven, A., and Bruckner, J. H.: 1957. The effect of nicarbazin on egg production and egg quality. Poultry Sci. 36:718.
Baldwin, F. M., Wiswell, O. B., and Jankewicz, H. A.: 1941. Hemorrhage control in *Eimeria tenella* infected chicks when protected by anti-hemorrhagic factor, Vitamin K. Proc. Soc. Exper. Biol. and Med. 48:278.
Becker, E. R.: 1933. Cross-infection experiments with coccidia of rodents and domesticated animals. Jour. Parasit. 19:230
———: 1934. Coccidia and Coccidiosis of Domesticated, Game, and Laboratory Animals and of Man. The Iowa State University Press, Ames, Iowa.
———: 1940. Coccidiosis of domesticated birds, with special reference to the common fowl. Vet. Med. 35:401.

———: 1956. Catalog of the Eimeriidae in genera occurring in vertebrates and not requiring intermediate hosts. Iowa St. Coll. Jour. Sci. 31:85.

———, Jessen, R. J., Pattillo, W. H., and Van Doorninck, W. M.: 1956. A biometrical study of the oocyst of *Eimeria necatrix*, a parasite of the common fowl. Jour. Protozool. 3:126.

———, and Zimmermann, W. J.: 1955. Influence of alcoholic extract of horse kidney on *Eimeria tenella* infection in chicks. Proc. Iowa Acad. Sci. 60:574.

———, Zimmermann, W. J., and Pattillo, W. H.: 1955. A biometrical study of the oocyst of *Eimeria brunetti*, a parasite of the common fowl. Jour. Protozool. 2:145.

———, Zimmermann, W. J., Pattillo, W. H., and Farmer, J. N.: 1956. Measurements of the unsporulated oocysts of *Eimeria acervulina*, *E. maxima*, *E. tenella*, and *E. mitis*: Coccidian parasites of the common fowl. Iowa St. Coll. Jour. Sci. 31:79.

Bejšovec, J.: 1960. Über die Passage der Kokzidiennoozysten durch den Verdauungstrakt der inadequaten Wirtstieren. Ceskoslov. Parasit. 7:217.

Berg, L. R., Hamilton, C. M., and Bearse, G. E.: 1951. The effect of coccidiosis (caused by *Eimeria maxima*) on egg quality. Poultry Sci. 30:298.

———, Hamilton, G. M., and Bearse, G. E.: 1956. The effect of furazolidone and other drug on the growth of chicks raised on cell litter containing coccidia. Poultry Sci. 35.876.

Bertke, E. M.: 1956. Pathological effects of coccidiosis caused by the protozoan parasite *Eimeria tenella* in chickens. Diss. Abstr. 16:183.

———, and Herrick, C. A.: 1954. The pathology of the kidney of the chicken produced by cecal coccidiosis. Jour. Parasit. 40 (No. 5, Sec. 2):30.

Boles, J. I., and Becker, E. R.: 1954. The development of *Eimera brunetti* Levine in the digestive tract of chickens. Iowa St. Coll. Jour. Sci. 29:1.

Boughton, D. C.: 1933. Diurnal gametic periodicity in avian Isospora. Am. Jour. Hyg. 18:161.

———: 1937a. Notes on avian coccidiosis. Auk 54:500

———: 1937b. Studies on oocyst production in avian coccidiosis. II. Chronic Isosporan infections in the sparrow. Am. Jour. Hyg 25:203.

———: 1939. Studies on the control of poultry coccidiosis. I. The sporulation of oocysts in various types of litter. Bul. Univ. Ga. Vol. 39. No. 8.

———, Boughton, R. B., and Volk, J. J.: 1938. Avian hosts of the genus Isospora (Coccidiida). Ohio Jour. Sci. 38:149.

———, and Volk, J. J.: 1938. Avian hosts of Eimerian coccidia. Bird Banding 9:139.

Brackett, S., and Bliznick, A.: 1949. The effect of small doses of drugs on oocyst production of infection with *Eimeria tenella*. Ann. N.Y. Acad. Sci. 52:595.

———, and Bliznick, A.: 1950. The Occurrence and Economic Importance of Coccidiosis in Chickens. Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.Y.

———, and Bliznick, A.: 1952a. The reproductive potential of five species of coccidia of the chicken as demonstrated by oocyst production Jour. Parasit. 38:133.

———, and Bliznick, A.: 1952b. The relative susceptibility of chickens of different ages to coccidiosis caused by *Eimeria necatrix*. Poultry Sci. 31:146.

Bradford, M. J., Herrick, C. A., and Wolfe, H. R.: 1947. Lethal effects of cecal contents from chickens infected with cecal coccidiosis and the inhibition of these effects with immune sera. Jour. Parasit. 33:393.

Bressler, G. O., and Gordeuk, S.: 1951. Effect of cecal coccidiosis on body weight, egg production and hatchability in chickens. Poultry Sci. 30.509.

Burns, W. C., and Challey, J. R.: 1959. Resistance of birds to challenge with *Eimeria tenella*. Exper. Parasit. 8:515.

Challey, J. R.: 1960. The effect of cecal coccidiosis infections and experimental hemorrhage upon adrenal ascorbic acid levels in the chicken. Jour. Parasit. 46:127.

———: 1962. The role of the bursa of Fabricius in adrenal response and mortality due to *Eimeria tenella* infections in the chicken. Jour. Parasit. 48:352.

———, and Burns, W. C.: 1959. The invasion of the cecal mucosa by *Eimeria tenella* sporozoites and their transport by macrophages. Jour. Protozool. 6:238.

Champion, L. R.: 1954. The inheritance of resistance to cecal coccidiosis in the domestic fowl. Poultry Sci. 33.670.

Clark, D. T., Smith, C. K., and Dardas, R. B.: 1962. Pathological and immunological changes in gnotobiotic chickens due to *Eimeria tenella*. Poultry Sci. 41:1635.

Cole, L. J., and Hadley, P. B.: 1910. Blackhead in turkeys: A study in avian coccidiosis. R.I. Sta., Bul. 141:137.

Corcurli, C.: 1928. Recherches sur la spécificité parasitaire des coccidies. Ann. Parasit. Hum. et Comp. 6:404.

Couch, J. R.: 1954. The chick hemorrhagic disease. Feedstuffs 26 (No. 16, April 17) 1:25.

Crooks, K. B. M.: 1934. Cross-infection experiments on parasite-free chicks with intestinal coccidia from the rabbit. Jour. Parasit. 20.277.

Cuckler, A. C., Garzillo, M., Malanga, C., and McManus, E. C.: 1960. Amprolium. 1. Efficacy for coccidia in chickens. Poultry Sci. 39:1241.

———, and Malanga, C. M.: 1955. Studies on drug resistance in coccidia. Jour. Parasit. 41:302.

Cuckler, A. C., and Malanga, C. M.: 1956 The effect of nicarbazin on the development of immunity to avian coccidia. Jour. Parasit. 42 593.

——, Malanga, C. M., Basso, A. J., and O'Neill, R. C.: 1955. The anti-parasitic activity of substituted carbanilide complexes Science 122.244.

——, Malanga, C. M., and Ott, W. H.: 1956. The anti-parasitic activity of nicarbazin. Poultry Sci. 35:98.

——, and Ott, W H: 1955. Tolerance studies on sulfaquinoxaline in poultry. Poultry Sci. 34.867.

——, Ott, W. H., and Fogg, D. E.: 1957. Factors in the evaluation of coccidiostats in poultry. Cornell Vet. 47:400.

Daugherty, J. W., and Herrick, C. A.: 1952. Cecal coccidiosis and carbohydrate metabolism in chickens. Jour. Parasit. 38:298.

Davies, S. F. M.: 1956. Intestinal coccidiosis in chickens caused by Eimeria necatrix. Vet. Rec. 68.853.

——: 1963. Eimeria brunetti, an additional cause of intestinal coccidiosis in the domestic fowl in Britain. Vet. Rec. 75:1.

——, and Joyner, L. P.: 1955. Observations on the parasitology of deep litter in poultry houses. Vet. Rec. 67:193.

——, and Joyner, L. P.: 1962. Infection of the fowl by the parenteral inoculation of oocysts of Eimeria tenella. Immunology London 194:996.

Delaplane, J. P., Batchelder, R. M., and Higgins, T C.: 1947. Sulfaquinoxaline in the prevention of Eimeria tenella infection in chickens. No. Am. Vet. 28:19.

——, and Higgins, T. C.: 1948. Sulfaquinoxaline in the prevention and control of chronic fowl cholera. Cornell Vet. 39:267.

——, and Stuart, H. O.: 1933. The common house fly as other than a simple mechanical carrier of avian coccidia. Poultry Sci. 12:390

——, and Stuart, H. O.: 1935 The survival of avian coccidia in soil Poultry Sci. 14:67.

Dickinson, E M.: 1939 The effects of variable dosages of sporulated Eimeria acervulina oocysts on chickens. Poultry Sci. 18 404.

——: 1941. The effect of variable dosages of sporulated oocysts of Eimeria acervulina oocysts on chickens. Poultry Sci. 20:413.

——: 1946 A factor in delayed production of Eimeria tenella oocysts. Poultry Sci. 25:391.

——: 1949 The effect of sulfaquinoxaline on Eimeria acervulina infection in pullets in egg production. Poultry Sci. 28 670.

——, Babcock, W. E., and Osebold, J. W.: 1951. Coccidial immunity studies in chickens. 1. Poultry Sci. 30 76.

Doran, D. J., and Farr, M. M.: 1962 Excystation of the poultry coccidium, Eimeria acervulina. Jour. Protozool. 9.154.

Duncan, S: 1959 The effects of some chemical and physical agents on the oocysts of the pigeon coccidium, Eimeria labbeana (Pinto, 1928). Jour. Parasit. 45:193.

Durant, A. J., and McDougle, H. C.: 1939. Coccidiosis in chickens and other birds. Univ. Mo. Agr. Exper Sta., Bull. 411.

Edgar, S. A.: 1954. Effect of temperature on the sporulation of oocysts of the protozoan, Eimeria tenella. Trans. Am. Micro Soc. 73 237.

——: 1955a. Sporulation of oocysts at specific temperatures and notes on the prepatent period of several species of avian coccidia. Jour. Parasit. 41:214.

——: 1955b. Planned immunization against cecal and intestinal coccidiosis, Am. Poultry Jour. Feb., 1955, p. 12.

——: 1955c. Effects of cecal coccidiosis (Eimeria tenella) on chickens of different ages, particularly during the early growing period. Poultry Sci. 34.1192

——: 1956. You can now inoculate against coccidiosis. Highlights Agr. Res. Vol. 3, No. 1.

——: 1960. Control "coxy" in layers. Poultry Tribune 66.29.

——: 1962. Coccidiosis of los pollos y guajolotes y su control por medio de la inmunización. Avicultura Moderna, Memorias XI Congreso Mundial Avicultura. P. 415.

——, King, D. F., and Johnson, L. W.: 1951. Control of avian coccidiosis through breeding or immunization. Poultry Sci. 30 911.

——, and Seibold, C. T.: 1964 A new coccidium of chickens, Eimeria mivati sp. n. (Protozoa: Eimeriidae) with details of its life history. Jour. Parasit. 50:193

Ellis, C. C.: 1938a. Part I. Studies of the viability of the oocysts of Eimeria tenella, with particular reference to conditions of incubation. Cornell Vet. 28:267.

——: 1938b. Part II. Studies on the effect of temperature on the sporulation time of Eimeria tenella. Cornell Vet. 28:272.

Fantham, H. B.: 1915 Coccidiosis in poultry and game birds. Jour. Bd. Agr. London. Vol. 21. No. 10.889.

Farr, M. M.: 1943. Resistance of chickens to cecal coccidiosis. Poultry Sci. 22:277.

——: 1963. Three new species of coccidia from the Canada goose. Poultry Sci. 22-277.

——: 1758). Jour. Wash. Acad. Sci. 43:336.

——, and Allen, R. W.: 1942. Sulfaguamidine feeding as a control measure for cecal coccidiosis of chickens. Jour. Am. Vet. Med. Assn 100-47.

———, and Doran, D. J.: 1962. Comparative encystation of four species of poultry coccidia. Jour. Protozool. 9:403.

———, and Wehr, E. E.: 1947. Developmental stages in the life cycle of Eimeria tenella affected by sulfamethazine treatment. Proc. Helminth. Soc. Wash. 14:2.

———, and Wehr, E. E.: 1949. Survival of Eimeria acervulina, E. tenella, and E. maxima oocysts on soil under various field conditions. Ann. N.Y. Acad. Sci. 52:463.

Fish, F.: 1931. The effect of physical and chemical agents on the oocysts of Eimeria tenella. Science 73:292.

Francis, D. W., and Shaffner, C. S.: 1956. An investigation of the morphological changes in young chickens and the reproductive performance of adult chickens fed furazolidone or nitrofurazone. Poultry Sci. 35:1371.

Gardiner, J. L.: 1954. Correlation of growth rate and severity of cecal lesions in chicks experimentally infected with cecal coccidiosis. Proc. Helminth. Soc. Wash. 21:82.

———: 1955. The severity of cecal coccidiosis infection in chickens as related to the age of the host and the number of oocysts ingested. Poultry Sci. 34:415.

———: 1957a. The effect of aureomycin and low-level sulfamethazine, separately and in combination, on cecal coccidiosis. Poultry Sci. 36:159.

———: 1957b. A comparison of the effect of aureomycin in combination with three levels of sulfamethazine in feed for the control of cecal coccidiosis of chickens. Jour. Parasit. 43.(No. 5, Sec. 2):17.

———: 1959. Control of experimental cecal coccidiosis with sulfaquinoxaline-antibiotic combinations. Poultry Sci. 38:1032.

———, and Farr, M. M.: 1954. Nitrofurazone for the prevention of experimentally induced Eimeria tenella infections in chickens. Jour. Parasit. 40:42.

———, Farr, M. M., and Wehr, E. E.: 1952. The coccidiostatic action of nitrophenide on Eimeria tenella. Jour. Parasit. 38:517.

———, and McLoughlin, D. K.: 1963. Drug resistance in Eimeria tenella. III. Stability of resistance to glycarbylamide. Jour. Parasit. 49:657.

Gill, B. S.: 1954a. Transmissibility of turkey coccidia (Eimeria meleagridis, E. melegrimitis and E. gallopavonis) to chickens. Indian Vet. Jour. 31:87.

———: 1954b. On the pathogenicity of Eimeria acervulina (Tyzzer 1929) to susceptible poultry. Indian Vet. Jour. 31:95.

———: 1954c. Speciation and viability of poultry coccidia in 120 fecal samples preserved in 2.5 percent potassium dichromate solution. Indian Jour. Vet. Sci. and Anim. Husb 21:243.

———, and Lall, N. B.: 1961. A fatal outbreak of Eimeria acervulina Tyzzer, 1929, in an experimental flock. Indian Jour. Vet. Sci. 31:315.

———, and Ray, H. N.: 1954. Glycogen and its possible significance in Eimeria tenella Railliet and Lucet, 1891. Indian Jour. Vet. Sci. and Anim. Husb. 24:223.

———, and Ray, H. N.: 1954b. On the occurrence of mucopolysaccharides in Eimeria tenella Railliet and Lucet, 1891. Indian Jour. Vet. Sci. and Anim. Husb. 24:229.

———, and Ray, H. N.: 1957. Life cycle and cytology of Eimeria tenella Railliet and Lucet, 1891 (Protozoa: Sporozoa), with notes on symptomatology and pathology of the infection. Mookerjee Mem. Volume (Proc. Zool. Soc., Calcutta):357.

Goble, F. C.: 1949. Para-substituted phenylarsonic acids as prophylactic agents against Eimeria tenella infections. Ann. N.Y. Acad. Sci. 52:533.

Goff, O. E.: 1945. Coccidiosis prevention and control in chickens by the use of sulphur. Timely Poultry Topics (La. St. Univ.), Vol. 3, No. 1.

Goodrich, H. P.: 1944. Coccidian oocysts. Parasit. 36:72.

Gordeuk, S., Bressler, G. O., and Glantz, P. J.: 1951. The effect of age of bird and degree of exposure in the development of immunity to cecal coccidiosis in chicks. Poultry Sci. 30:503.

Graven, U.: 1955. Zur Pathologie der Geflügelcoccidiose. Arch. Protistenk. 98:342.

Grumbles, L. C., and Delaplane, J.: 1948. Relative acutity of sulfamethazine and sulfaquinoxaline against Eimeria tenella infection in young chickens. Am. Jour. Vet. Res. 9:306.

———, Delaplane, J. P., and Higgins, T. C.: 1948. Continuous feeding of low concentrations of sulfaquinoxaline for the control of coccidiosis in poultry. Poultry Sci. 27:605.

———, Tower, B. A., Oglesby, W. T., and Upp, C. W.: 1949. Field observations on the use of sulfaquinoxaline for the control of coccidiosis in young chickens. Ann. N.Y. Acad. Sci. 52:558.

Grzimek, B.: 1931. Jodmilch gegen Kuckenkokzidiose. Arch. f. Geflügelk. 5 287.

Haase, A.: 1939. Untersuchungen über die bei deutschen Wildhühnern vorkommenden Eimeria-Arten. Arch. Protistenk. 92:329.

Hadley, P. B.: 1910. Studies on avian coccidiosis. I. III. Coccidiosis in the English sparrow and other wild birds. Zentralbl. f. Bakt. I. Orig. 56:522.

Harms, R. H., and Tugwell, R. L.: 1956. The effect of experimentally induced prolonged blood clotting time on cecal coccidiosis of chicks. Poultry Sci. 35:937.

Harwood, P. D., and Stunz, D. I.: 1949a. Nitrofurazone in the medication of avian coccidiosis. Jour. Parasit. 35:175.

———, and Stunz, D. I.: 1949b. Nitrofurazone and coccidiosis. Ann. N.Y. Acad. Sci. 52:558.

———, and Stunz, D. I.: 1950. The efficary of nitrofurazone led continuously for the control of avian coccidiosis under conditions of natural infection. Proc. Helminth. Soc. Wash. 17:103.

Harwood, P. D., and Stunz, D. I.: 1953. A search for drug-fast strains of *Eimeria tenella*. Jour. Parasit. 39:268.

——, and Stunz, D. I.: 1954. Efficacy of furazolidone, a new nitrofuran, against blackhead and coccidiosis. Jour. Parasit. 40 (No. 5, Sec. 2):21.

——, Stunz, Dale I., and Wolfgang, R. W.: 1957. Furazolidone in the treatment of coccidiosis. Jour. Parasit. 43 (No. 5, Sec. 2):18

Hawkins, P. A.: 1949. Sulfamethazine treatment of cecal coccidiosis. Poultry Sci. 22:459.

——: 1952. Coccidiosis in turkeys. Mich. State Coll. Agr. Exper. Sta., Tech. Bul. 226.

——, and Dunlap, J. S.: 1949. Bisphenols for the control of cecal coccidiosis. Poultry Sci. 28:818.

——, and Kline, E. E.: 1945. The treatment of cecal coccidiosis with sulfamethazine. Poultry Sci. 24:277

——, and Rausch, R.: 1946. Sodium sulfamerazine in the treatment of cecal coccidiosis. Poultry Sci. 25:181.

Hegner, R. W.: 1923. The effects of changes in diet on the incidence, distribution, and numbers of certain intestinal protozoa of rats. Am. Jour. Hyg. 3:180.

——: 1924. The relations between a carnivorous diet and mammalian infections with intestinal protozoa. Am. Jour. Hyg. 4:393.

——, and Andrews, J. M.: 1925. Effects of a carnivorous diet on the intestinal pH of rats with reference to flagellates. Am. Jour. Hyg. 5:357.

Henry, D. P.: 1931. Species of coccidia in chickens and quail in California. Univ. Calif. Pub. Zool. 36:157

Herrick, C. A.: 1934. The development of resistance to the protozoan parasite, *Eimeria tenella*. Jour. Parasit. 20:329.

——, and Holmes, C. E.: 1936. Effects of sulphur on coccidiosis in chickens. Vet. Med. 31:390.

——, Holmes, C. E., and Degusti, D. L.: 1942. The experimental use of organic sulfur compounds for the prevention of cecal coccidiosis in chickens. Am. Jour. Vet. Res. 3:117.

——, Ott, G. L., and Holmes, C. E.: 1936a. Age as a factor in the development of resistance of the chicken to the effects of the protozoan parasite, *Eimeria tenella*. Jour. Parasit. 22:264.

——, Ott, G. L., and Holmes, C. E.: 1936b. The chicken as a carrier of the oöcysts of the coccidia, *Eimeria tenella*. Poultry Sci. 15:322.

Holmes, C. E., Deobald, H. J., and Herrick, C. A.: 1938. Sulphur and rickets. Poultry Sci. 17:136

Hotton Smith, C.: 1942. Sulphoguanidine in avian coccidiosis. Vet. Record 54:259.

——: 1947. Coccidiosis—some factors influencing its epidemiology. Vet. Record 59:645.

——: 1948. The effects of sulphonamides on coccidiosis of poultry caused by *Eimeria tenella*. Proc. 8th World's Poultry Cong. Copenhagen 1,732.

——: 1949. Some factors influencing the origin and course of epidemics of coccidiosis in poultry. Ann. N.Y. Acad. Sci. 52:449.

——: 1954. Parasitology of deep litter. Agr.: The Jour. of the Ministry of Agr. (Great Britain) 60:569.

——: 1957a. Additives for disease control in poultry and turkeys. Vet. Record 69(suppl):164.

——: 1957b. Factors affecting the transmission of coccidia and the development of disease in fowls. P. 35, Biol. Aspects of the Transmission of Disease. Oliver and Boyd, Edinburgh and London.

——, Beattie, J., and Long, P. L.: 1961. Resistance to *Eimeria tenella* and its transference from one cecum to the other in individual fowls. Immunol. 4:111.

——, and Boyland, E.: 1946. Sulphonamides in the treatment of caecal coccidiosis of chickens. Brit. Jour. Pharm. and Chem. 1,139

——, and Long, P. L.: 1952. Nitrofurazone in the treatment of caecal coccidiosis of chickens. Brit. Vet. Jour. 108:47.

——, and Long, P. L.: 1959a. The effects of different anticoccidial agents on the intestinal coccidiosis of the fowl. Jour. Comp. Path. and Therap. 69:192.

——, and Long, P. L.: 1959b. The anticoccidial activity of glycarbylamide. Brit. Vet. Jour. 115:55.

——, and Long, P. L.: 1963. Coccidia and coccidiosis in the domestic fowl and turkey. Advances in Parasitology. Vol. 1, ed. Dawes, B. Academic Press, London and New York, Pp. 67-107.

——, Long, P. L., and Pierce, A. E.: 1963. Behavior of invasive stages of *Eimeria tenella* in the immune fowl. Exper. Parasit. 14:66

——, and Taylor, E. L.: 1942. Sulphamethazine and sulphadiazine treatment in caecal coccidiosis of chickens. Vet. Record 54:516.

——, and Taylor, E. L.: 1943. Saturated solution of sulphamethazine as a substitute for drinking water in the treatment of caecal coccidiosis in chickens. Vet. Record 55:109.

——, and Taylor, E. L.: 1945. Sulphamethazine in the drinking water as a treatment for caecal coccidiosis in chickens. Vet. Record 57:35.

——, Taylor, E. L., and Turtle, E. E.: 1940. Ammonia fumigation for coccidial disinfection. Vet. Record 52:829.

Ikeda, M.: 1955. Factors necessary for *E. tenella* infection of the chicken. II. Influence of the pancreatic juice on infection. Jap. Jour. Vet. Sci. 17:225.

———: 1960. Factors necessary for *E. tenella* infection of the chicken VI. Excystation of the oocyst *in vitro*. Jap. Jour. Vet. Sci. 22:27.

Itagaki, K.: 1952. Studies on the infectious process of coccidium in fowl. I. Sporogony and external conditions. Jap. Jour. Vet. Sci. 14:237.

———: 1954. Further investigation on the mechanism of coccidial infection in fowl. Jour. Fac. Agric. Tottori Univ. 2:37.

———, and Tsubokura, M.: 1953. Studies on the infectious process of coccidium in fowl. III. On the liberation of sporozoite from oocyst. Jap. Jour. Vet. Sci. 15:1.

———, and Tsubokura, M.: 1954. Studies on coccidiosis in fowls. III. On the hemolysis by merozoite. Jap. Jour. Vet. Sci. 16:159.

Jankiewicz, H. A., and Scofield, R. H.: 1934. The immunization of chicks to caecal coccidiosis under conditions of poor sanitation. Los Angeles County Livestock Dept., Los Angeles, Calif.

Johansson, K. R., and Sarles, W. B.: 1948. Bacterial population changes in the ceca of young chickens infected with *Eimeria tenella*. Jour. Bacteriol. 56:635.

Johnson, J. E., Mussell, D. R., and Dietzler, A. J.: 1949. The activity of 4,4' isopropylidenebis (2-isopropylphenol) on cecal coccidiosis (*Eimeria tenella*) in chickens. Ann. N.Y. Acad. Sci. 52:518.

Johnson, W. T.: 1923. Avian coccidiosis. Poultry Sci. 2:146.

———: 1931. Effect of five species of *Eimeria* upon egg production of Single Comb White Leghorns. Jour. Parasit. 18:122.

———: 1932. Immunity to coccidiosis produced by inoculation through the ration. Jour. Parasit. 19:160.

———: 1938. Coccidiosis of the chicken with special reference to species. Ore. Agr. Exper. Sta., Bul. 358.1.

Joyner, L. P.: 1958. Experimental *Eimeria mitis* infections in chickens. Parasit. 48:101.

———: 1960. The relationship between toxicity and coccidiostatic efficacy of pyrimethamine and sulphonamides and their relative reversal by folic acid. Research Vet. Sci. 1:2

———, and Davies, S. F. M.: 1960. Detection and assessment of sublethal infections of *Eimeria tenella* and *Eimeria necatrix*. Exper. Parasit. 9:243

———, and Kendall, S. B.: 1955. Synergism in the chemotherapy of *Eimeria tenella*. Nature 176:975.

Jungherr, E. L., and Winn, J. D.: 1949. Continuous low level sulfaquinoxaline feeding in the practical control of coccidiosis in broilers. Ann. N.Y. Acad. Sci. 52:563.

Kendall, S. B., and Joyner, L. P.: 1956. The potentiation of coccidiostatic drugs by pyrimethamine. Vet. Record 68:119.

Kennard, D. C., and Chamberlin, V. D.: 1947. Lime treatment of floor litter for chickens. Bimonthly Bul., Ohio Agr. Exper. Sta. 32:11.

Kerr, W. R., and Botham, G. H.: 1935. Iodine in the control and treatment of avian coccidiosis. Vet. Jour. 87:10.

———, and Common, R. H.: 1955. The effects of certain acid treatments for coccidiosis on the H ion content of the fowl's intestine. Vet. Jour. 91:309

King, D. F., Edgar, S. A., and Johnson, L. W.: 1951. Breeding and immunizing chickens for resistance to coccidiosis. 60 and 61. Ann. Repts. Ala. Agr. Exper. Sta., pp. 49–50.

Kogan, Z. M.: 1959. Survival of the sporulated and unsporulated oocysts of chick coccidiae under the hibernation in different conditions. Zool. Zhurnal 38.684.

———: 1960. Survival of chick coccidia oocysts after repeated hibernation under natural conditions in the Byelorussia. Zool. Zhurnal. 39.617.

Koutz, F. R.: 1948. Immunity studies in avian cecal coccidiosis. II. The use of deep litter and hydrated lime treated litter in the development of immunity in young chickens. Poultry Sci. 27:793.

———: 1950. The survival of avian coccidia in the soil. The Speculum (Ohio St. Univ., Coll Vet. Med.) 3:1.

———: 1952a. The effect of built-up litter on the parasite ova and oocysts of poultry. Poultry Sci. 31:123.

———: 1952b. The problem of poultry parasites in deep and built up litter. The Speculum (Ohio St. Univ., Coll. Vet. Med.) 5(3):18.

Krassner, S. M.: 1963. Factors in host susceptibility and oocyst infectivity in *Eimeria acervulina* infections. Jour. Protozool. 10:327.

Krijgsman, B. J.: 1929a. Übertragung und Prophylaxis der Kokzidiose. Zentralbl I. Bakt. I. Orig. 111:438.

———: 1929b. Durch Protozoen verursachte Krankheiten. *In* van Heelsbergen, T.: Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart. Pp. 350–73.

Levine, L., and Herrick, C. A.: 1954. The effects of the protozoan parasite *Eimeria tenella* on the ability of the chicken to do muscular work when its muscles are stimulated directly and indirectly. Jour. Parasit. 40.525.

———, and Herrick, C. A.: 1957. Effects of cecal coccidia on the ability of Plymouth Rocks to do muscular work. Poultry Sci. 36.24.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and Man. Burgess Publishing Company, Minneapolis, Minn. 412 pp

Levine, P. P.: 1938. Eimeria hagana n. sp. (Protozoa : Eimeriidae) a new coccidium of the chicken. Cornell Vet. 28 263.

———: 1939. The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. 29:309.

———: 1940a. Sub-clinical coccidial infection in chickens. Cornell Vet. 30:127.

———: 1940b. The effect of sulfapyridine on experimental avian coccidiosis. Jour. Parasit. 26:233.

———: 1940c. The initiation of avian coccidial infection with merozoites. Jour. Parasit. 26:337.

———: 1941a The coccidiostatic effect of sulfaguanidine (sulfanilylguanidine). Cornell Vet. 31:107.

———: 1941b. Chemotherapy in the control of avian coccidiosis. Proc. U.S. Livestock San. Assn., 1941.

———: 1942a The periodicity of oocyst discharge in coccidial infections of chickens. Jour. Parasit. 28 346

———: 1942b. Excystation of coccidial oocysts of the chicken. Jour. Parasit. 28:426.

———: 1942c. A new coccidium pathogenic for chickens, Eimeria brunetti n. sp. (Protozoa: Eimeriidae), Cornell Vet. 32:430.

———: 1945. Specific diagnosis and chemotherapy of avian coccidiosis. Jour. Am. Vet. Med. Assn. 106 68

Long, P. L 1959 A study of Eimeria maxima Tyzzer, 1929, a coccidium of the fowl (Gallus gallus) Ann. Trop. Med. and Parasit. 53:325.

———: 1962 Observations on the duration of the acquired immunity of chickens to Eimeria maxima Tyzzer, 1929 Parasit. 52:89.

———, and Binstead, J. A.: 1959. Observations on mixed parasitic infections acquired by chickens reared on old built-up litter. World's Poultry Sci. Jour. 15:353.

———, and Rootes, D. G.: 1959 Cytochemical studies of Eimeria in the fowl. Trans. Roy. Soc. Trop. Med and Hyg. 53 308.

———, and Rose, M. E.: 1962. Attempted transfer of resistance to Eimeria tenella infections from domestic hens to their progeny. Exper. Parasit. 12:75.

Lux, R. E: 1954 The chemotherapy of Eimeria tenella. I, Diaminopyrimidines and dihydrotriazenes Antibiotics and Chemotherapy 4.971.

McDermott, J. J., and Stauber, L. A.: 1954. Preparation and agglutination of merozoite suspensions of the chicken coccidian, Eimeria tenella. Jour. Parasit. 40 (No. 5, Sec. 2) :23.

McEvey, G. F.: 1955 The restriction of ooporphyrin deposition on egg shells by drug feeding. Poultry Sci. 34:1164.

McLoughlin, D. K., and Chester, D. K.: 1959. The comparative efficacy of six anticoccidial compounds. Poultry Sci. 38:353.

———, and Gardiner, J. L 1961. Drug-resistance in Eimeria tenella. II. The experimental development of a glycarbylamide-resistant strain. Jour. Parasit. 47:1001.

———, and Gardiner, J. L.: 1962a. Drug resistance in Eimeria tenella. II. The experimental development of a zoalene resistant strain. Jour. Parasit 48.341.

———, and Gardiner, J. L.: 1962b. The activity of amprolium in Eimeria tenella infections-laboratory trials Avian Dis. 6:185.

———, Gardiner, J. L., and Chester, D. K.: 1960. The activity of glycarbylamide, trithiadol, and nicarbazin against Eimeria tenella in chickens Poultry Sci. 39:1328.

———, Rubin, R., and Cordray, D. R.: 1957. The development of immunity to ceral coccidiosis in the presence of nicarbazin. Preliminary report. Poultry Sci. 36:1003.

Mayhew, R. L.: 1932a Studies on coccidiosis. I. The effects of coccidiosis upon the weights of chickens artificially inoculated during the seventh week. Poultry Sci. 11:34.

———: 1932b. Studies on coccidiosis II. The effects of coccidiosis upon the weights of chickens artificially inoculated during the thirteenth and fourteenth weeks Poultry Sci. 11:102.

———: 1932c. Studies on coccidiosis. III. Observations on paralysis with special reference to coccidial infection. Poultry Sci. 11,289.

———: 1933 Studies on coccidiosis. IV. Mortality and infection among artificially inoculated chickens Poultry Sci. 12-206.

———: 1934a. Studies on coccidiosis V. Treatment with powdered buttermilk. Jour. Parasit. 20:230.

———: 1934b. Studies on coccidiosis VI. Effect of early attack on egg production. Poultry Sci. 13:148.

———: 1934c. Studies on coccidiosis. VII. Effects of starvation and removal of caeca. Poultry Sci. 13:360.

———: 1934d Studies on coccidiosis VIII. Immunity or resistance to infection in chickens. Jour. Am. Vet. Med. Assn. 85:729.

———: 1934e. Some practical results of experiments on coccidiosis in chickens. La. Sta., Circ. 7:1.

———: 1937. Studies on coccidiosis. IX. Histopathology of the caecal type in the chicken. Trans. Am. Micr. Soc. 56:431.

Metelkin, A.: 1935. The role of flies in the spread of coccidioses in animals and man. Med. Parasit. and Parasit. Dis. (Moscow) 4:75–82. Abst. in Trop. Dis Bul. 32.660.

Monné, L., and Honig, G.: 1954. On the properties of the shells of the coccidian oocysts. Ark. Zool., Stockholm, 2. s. 7:251.

Morehouse, N. F.: 1938. The reaction of the immune intestinal epithelium of the rat to reinfection with *Eimeria nieschulsi*. Jour. Parasit. 24:311.

———: 1946. The effect of some halogenated arsonic acids and their sodium salts on *Eimeria tenella* infection in chickens. Jour. Parasit. (Dec. suppl.) 32:8.

———, and Mayfield, O. J.: 1946. The effect of some aryl arsonic acids on experimental coccidiosis infection in chickens. Jour. Parasit. 32:20.

———, and McGuire, W. C.: 1956. Morbidity and mortality among chickens infected with large numbers of the intestinal coccidium, *Eimeria acervulina*. Jour. Parasit. 42 (No. 4, Sec. 2):24.

———, and McGuire, W. C.: 1959. The use of 3, 5-dinitrobenzamide and its N-substituted derivatives against coccidiosis in chickens. Poultry Sci. 38:410.

Moynihan, I. W.: 1950. The role of the protozoan parasite, *Eimeria acervulina*, in disease of the domestic chickens. Canad. Jour. Comp. Med. 14:74.

Natt, M. P.: 1959. The effect of cecal coccidiosis on the blood cells of the domestic fowl. 3. The changes in the leucocyte picture during the course of infection. Exper. Parasit. 8:182.

———, and Herrick, C. A.: 1955. The effect of cecal coccidiosis on the blood cells of the domestic fowl. 1. A comparison of the changes in the erythrocyte count resulting from hemorrhage in infected and mechanically bled birds. The use of the hematocrit value as an index of the severity of the hemorrhage resulting from the infection. Poultry Sci 34:1100.

———, and Herrick, C. A.: 1956. The effect of cecal coccidiosis on the blood cells of the domestic fowl. 2. The changes in the blood volume during the course of the infection. Poultry Sci. 35:311.

Newberne, P. M., and Buck, W. B.: 1956. Studies on drug toxicity in chicks. 2. The influence of various levels of sulfaquinoxaline on growth and development of chicks. Poultry Sci. 35:1259.

———, and Buck, W. B.: 1957. Studies on drug toxicity in chicks. 3. The influence of various levels of nicarbazin on growth and development of chicks. Poultry Sci. 36.304.

———, and McDougle, H. C.: 1956. Studies on drug toxicity in chicks. 1. The influence of various levels of megasul (nitrophenide, M,M¹ dinitrodiphenyl disulfide) on growth and development of chicks. Poultry Sci. 35:1044.

———, and McEuen, C. L.: 1957. Studies on drug toxicity in chicks 4. The influence of various levels of nitrofurazone on growth and development of chicks. Poultry Sci. 36:759.

Ott, W. H., Kuna, S., Porter, C. C., and Cuckler, A. C.: 1955. Biological studies of nicarbazin, a new anticoccidial agent. Poultry Sci. 34:1215.

———, Kuna, S., Porter, C. C., Cuckler, A. C., and Fogg, D. E.: 1956. Biological studies on nicarbazin, a new anticoccidial agent. Poultry Sci. 35:1355.

Otto, G. F., Jeske, H. A., Frost, D. V., and Perdue, H. S.: 1958. Menadione so···m bisulfite complex (Klotogen F) in cecal coccidiosis. Poultry Sci. 37.201.

Patterson, F. D.: 1933. Cross infection experiments with coccidia of birds. Cornell Vet. 23.249

Pattillo, W. H.: 1959. Invasion of the cecal mucosa of the chicken by sporozoites of *Eimeria tenella* and *E. necatrix*. Iowa St. Coll. Jour. Sci. 31:492.

———: 1959. Invasion of the cecal mucosa of the chicken by sporozoites of *Eimeria tenella* Jour. Parasit. 45.253.

———, and Becker, E. R.: 1955. Cytochemistry of *Eimeria brunetti* and *E. acervulina* of the chicken. Jour. Morph. 96 61.

Pellérdy, L.: 1961. *Eimeria brunetti* und Enddarmkokzidiose in Ungarn. Angewandte Parasit. 2:77.

———: 1962. Über die Spezifität der in verschiedenen Galliformes schmarotzenden Arten der Gattung Eimeria. Acta Vet. Acad. Sci. Hungar. 12.279.

Pérard, C. H.: 1925. Recherches sur les coccidies et les coccidimes du lapin. Ann. de l'Inst. Pasteur 39.505.

———: 1933. Sur le rôle des espèces non sensibles dans la propagation des coccidioses. Bul. Acad. Vétér. de France 86:206.

Peterson, E. H.: 1948. The effect of sulfaquinoxaline medication on *Eimeria tenella* infection in chickens. Am. Jour. Vet. Res. 9:77.

———: 1949. Coccidiosis in laying hens due presumably to *Eimeria acervulina*. Am. N.Y. Acad. Sci. 52:464.

———: 1960. A study of anticoccidial drugs against experimental infections with *Eimeria tenella* and *necatrix*. Poultry Sci. 39:759.

———, and Hymas, T. A.: 1950. Sulfaquinoxaline, nitrofurazone and nitrophenide in the prophylaxis of experimental *Eimeria necatrix* infection. Am. Jour. Vet. Res. 11:278.

———, and Munro, S. S.: 1949. The chemotherapy of coccidiosis due to *Eimeria acervulina*. Ann. N.Y. Acad. Sci. 52:579.

Pierce, A. E., Long, P. L., and Horton-Smith, C.: 1962. Immunity to *Eimeria tenella* in young fowls (*Gallus domesticus*). Immunol. 5:129.

Pierce, A. E., Long, P. L., and Horton-Smith, C. 1963 Attempts to induce a passive immunity to *Eimeria tenella* in young fowls (*Gallus domesticus*) Immunol. 6:37.

Polin, D., Ott, W. H., and Siegmund, O. H.: 1956. Observations on mottled egg yolks. Feedstuffs 28 (No. 23, June 9):18

——, Ott, W. H., and Siegmund, O. H.: 1957. The incidence and degree of yolk mottling in eggs from hens fed diets with and without nicarbazin. Poultry Sci. 36:524.

Pratt, I.: 1937. Excystation of the coccidia, *Eimeria tenella*. Jour. Parasit. 23:426.

——: 1940 The effect of *Eimeria tenella* (coccidia) upon the blood sugar of the chicken. Trans Am. Micr Soc 59 31.

——: 1941 The effect of *Eimeria tenella* upon the glycogen stores of the chicken. Am. Jour. Hyg 31 51

Ray, H N 1945 On a new coccidium IFenyonella gallinae n, sp. from the gut of the domestic fowl, *Gallus gallus domesticus* Linn. Current Sci. 14 275.

——, and Gill B S 1954 Preliminary observations on alkaline phosphatase in experimental *Eimeria tenella* infection in chicks. Ann. Trop. Med. and Parasit. 48.8.

——, and Gill, B S : 1955 Observations on the nucleic acids of *Eimeria tenella* Railliet and Lucet, 1891 Indian Jour Vet. Sci and Anim Husb. 25·17.

Reid, W. M , Sharma, N. N., and Reener, J : 1961. Intestinal species of coccidia in chickens from Georgia Jour Parasit. 47(suppl.):45.

Ripsom, C A and Elerrick, C A, 1945 Effects of various sulfa compounds on the protozoan parasite, *Eimeria tenella*. Jour Parasit. 31 98

Rose, M E , and Long, P. L: 1962 Immunity to four species of *Eimeria* in fowls. Immunol. 5.79

Rosenberg, M. M , Alicata, J E., and Palafox, A. L : 1954. Further evidence of hereditary resistance and susceptibility to cecal coccidiosis in chickens. Poultry Sci, 33:972.

Roudabush, R L : 1937. The endogenous phases of the life cycles of *Eimeria nieschulzi, E. separata,* and *L. miyatmi,* coccidian parasites of the rat. Iowa St. Coll. Jour. Sci. 11:135.

Rubin, R., McLoughlin, D. K., Costello, L. C., and Wehr, E. E. 1956. The efficacy of nicarbazin as a prophylactic drug in cecal coccidiosis of chickens. Poultry Sci. 35,856.

Sawyer, C. E, and Hamilton, C. M.: 1935. Coccidiosis in chickens Poultry Pointers No. 6 (Revised) St. Coll, of Wash. Ext. Serv.

Schildt, C. D., and Herrick, C. A.: 1955 The effect of cecal coccidiosis on the motility of the digestive tract of the domestic fowl. Jour. Parasit. 41 (No. 6, Sec. 2):18.

Scholtyseck, F· 1953. Beitrag zur Kenntnis der Entwicklungsganges der Hühnercoccids *Eimeria tenella*, Arch. Protistenk. 98.415.

——: 1954 Untersuchungen über die bei einheimischen Vogelarten vorkommenden Coccidien der Gattung Isospora. Arch.f Protistenk. 100 91.

——· 1956 Untersuchungen über Coccidieninfektion bei Vögeln. Zentralbl. Bakt., I Abt. Orig. 165 275.

——: 1959. Zur Pathologie der *Eimeria-maxima-Coccidiose*, Zentralbl. Bakt., I Abt. Orig. 175:303.

——, and Weissenfels, N · 1956. Elektronenmikroskopische Untersuchungen von Sporozoen. 1. Die Oocystenmembran des Hühnercoccids *Eimeria tenella*. Arch. Protistenk, 101:215.

Schwalbach, G · 1959 Untersuchungen und Beobachtungen an Coccidien der Gattung *Eimeria, Isospora* und *Caryospora* bei Vögeln mit einer Beschreibung von sechzehn neuen Arten. Arch. Protistenk. 104:431.

——: 1960. Die Coccidiose der Singvögel. I. Der Ausscheidungsrhythmus der *Isospora*-Oocysten beim Hausperling (*Passer domesticus*). Zentralbl. Bakt., I Abt., Orig. 178.263.

——: 1961a: Die Coccidiose der Singvögel. II. Beobachtungen an *Isospora*-Oocysten aus einem Weichfresser (*Parus major*) mit besonderer Berücksichtigung des Ausscheidungsrhythmus. Zentralbl. Bakt., I Abt., Orig. 181.261.

——: 1961b: Die Coccidiose der Singvögel. III. Die Temperaturabhängigkeit der exogenen Entwicklungsphase. Zentralbl. Bakt., 1 Abt., Orig. 181.272.

Seeger, K. C., and Tomhave, A. E.: 1916 Effect of sulfaguanidine on caecal coccidiosis. Del. Agr. Exper. Sta, Bul. 260.

Sharma, N N., and Reid, W M. 1962. Successful infection of chickens after parenteral inoculation of *Eimeria* spp. Jour. Parasit. 48(April):53.

Sherwood, D. H , Milby, T T., and Higgins, W A.: 1956. The effect of nicarbazin on reproduction in White Rock breeder hens. Poultry Sci. 35:1014.

Smith, B. F., and Herrick, C. A : 1944. The respiration of the protozoan parasite, *Eimeria tenella*. Jour Parasit. 30.295.

Smith, T., and Smillie, E. W.: 1917. Note on coccidia in sparrows and their assumed relation to blackhead in turkeys. Jour. Exper Med 26 415.

Steward, J. S.: 1947 Host-parasite specificity in coccidia. Infection of the chicken with the turkey coccidium, *Eimeria meleagridis*. Parasit. 38:157.

Stuart, E. E., Bruins, H. W., and Keenum, R. D.: 1963. The immunogenicity of a commercial coccidiosis vaccine in conjunction with trithiadol and zoalene. Avian Dis. 7:12.

Swales, W. E.: 1944. On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. Canad. Jour. Res., D, 22:131.

———: 1946a. On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. II. Further studies on the use of drugs in established infections. Canad. Jour. Comp. Med. and Vet. Sci. 10:3.

———: 1946b. The chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens IV. Experiments on the use of chemotherapy during the immunizing exposure of chicks. Jour. Am. Vet. Med. Assn. 108:393.

———: 1947a. New methods of controlling caecal coccidiosis in chicks. Canad. Jour. Comp. Med. and Vet. Sci. 11:5.

———: 1947b. Method of controlling caecal coccidiosis of chicks. Dom. of Canada, Dept. Agr. Publ. 788, Farmer's Bul. 14f.

———: 1950. On the chemotherapy of cecal coccidiosis (*Eimeria tenella*) of chickens. VII. The use of a standardized test to determine coccidiostatic properties of drugs. Canad. Jour. Comp. Med. 14:269.

———: 1951. On the chemotherapy of caecal coccidiosis (*Eimeria tenella*) of chickens. VIII. Field trials of broiler feeds medicated at a low level. Canad. Jour. Comp. Med. 15:125.

Tienhoven, A. van, Crawford, R. D., and Duchaine, S. A.: 1957. The effect of nicarbazin on spermatogenesis and semen quality. Poultry Sci. 36 760.

Tsunoda, K., and Itikawa, O.: 1955. Histochemical studies of chicken coccidia (*Eimeria tenella*), I. On the nucleic acids, polysaccharides and phosphomonoesterases in their several developmental stages. Govt. Exper. Station Animal Hyg., Tokyo, Exper. Rep. 29, p. 79.

Tugwell, R. L.: 1955. The relative activity of selected drugs used as coccidiostats. Poultry Sci. 34:1368.

Tyzzer, E. E.: 1929. Coccidiosis in gallinaceous birds. Am. Jour. Hyg 10:269.

———: 1932. Criteria and methods in the investigation of avian coccidiosis. Science 75:324.

———, Theiler, H., and Jones, E. E.: 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of *Eimeria* of the chicken. Am. Jour. Hyg. 15:319.

———: 1937. A discussion of factors influencing the course of coccidiosis. Jour. Am. Vet. Med. Assn. 90 341.

Uricchio, W. A.: 1953. The feeding of artificially altered oocysts of *Eimeria tenella* as a means of establishing immunity to cecal coccidiosis in chickens. Proc. Helminth. Soc. Wash. 20 (2):77.

Van Doorninck, W. M., and Becker, E. R.: 1957 Transport of sporozoites of *Eimeria necatrix* in macrophages. Jour. Parasit. 43 40.

Van Es, L., and Olney, J. F.: 1937. The evolution of a sanitary type of chick feeder. Nebr. Agr. Exper. Sta., Bul. 806rl.

——— and Olney, J. F.: 1940. An inquiry into the nature of environment on the incidence of poultry diseases. Nebr. Agr. Exper. Sta., Res. Bul. 118.

Venard, C.: 1933. Helminths and coccidia from Ohio bobwhite. Jour. Parasit. 19:205.

Waletzky, E., and Hughes, C. O.: 1946. The relative activity of sulfanilamides and other compounds in avian coccidiosis (*Eimeria tenella*). Am. Jour. Vet. Res. 7:365.

———, Hughes, C. O., and Brandt, M. C.: 1949. The anti-coccidial activity of nitrophenide. Ann. N.Y. Acad. Sci. 52:543.

Warner, D. E.: 1933. Survival of coccidia of the chicken in soil and on the surface of eggs. Poultry Sci. 12 343.

Waxler, S. H.: 1941a. The effect of feeding concentrated physiological saline to chickens during cecal coccidiosis. Trans. Am. Micr. Soc. 60:453.

———: 1941b. Immunization against cecal coccidiosis in chickens by the use of X-ray attenuated oocysts. Jour. Am. Vet. Med. Assn. 99.481.

Wehr, E. E., and Farr, M. M.: 1947. Effect of sulfamethazine on the coccidian parasite, *Eimeria tenella*, of chickens. Proc. Helminth. Soc. Wash. 14:1.

Weiss, H. S.: 1957. Further comments on the effect of nicarbazin on the egg. Poultry Sci. 36:589.

Wellman, G.: 1954. Beitrag zur Frage der Infektionsweg der Hühnerkokzidiose. Zeitschr. Ang. Zool. 41:139.

West, J. L.: 1940. Coccidiosis of domesticated animals and fowls. Jour. Am. Vet. Med. Assn. 96.603.

Wiley, J. R.: 1956. Coccidiosis still a threat to your pullets. Canad. Poultryman 43:22.

Wilson, P. A. G., and Fairbairn, D.: 1961. Biochemistry of sporulation in oocysts of *Eimeria acervulina*. Jour. Protozool. 8.410.

Wolfgang, R. W., Stunz, D. I., and Harwood, P. D.: 1957. The effect of furazolidone administered in the feed upon coccidiosis. Jour. Parasit. 43 (No. 5, Sec. 2) :17.

Yakimoff, W. L., and Iwanoff-Gobzem, P. S.: 1931. Zur Frage der Infektion der Tiere mit heterogenen Kokzidien. Zentralbl. f. Bakt. I. Orig 122 319.

———, Iwanoff-Gobzem, P. S., and Buewnsch, B. L.: 1932. Zur Frage der Infektion der Tiere mit heterogenen Kokzidien. II. Mitteilung. Zentralbl. f. Bakt. I. Orig. 125:469.

turkey poults. Hawkins (1952) considered it to be the cause of the most serious coccidial infection of turkeys. With a dose of 50,000 oocysts he was able to produce up to 100 per cent mortality in poults two to three weeks of age. He mentioned that there was some evidence of age resistance. Clarkson and Gentles (1958) confirmed this observation and stated that "although age resistance develops early, reduction in weight gains occurs in previously uninfected birds of all ages and can cause severe economic loss."

There are no symptoms characteristic of this infection. According to Hawkins (1952) and Clarkson and Gentles (1958) the birds appear normal until the end of the fourth day after infection when they begin to cheep and huddle together with drooping wings and ruffled feathers. The feces become fluid and contain some flecks of blood. Hawkins (1952) stated that cylindrical pellets, two to three centimeters long, appear in the feces on the fifth and sixth days after infection, whereas Clarkson and Gentles (1958) reported that on the sixth day, the feces consist "of a foul-smelling, dark brown liquid material containing small streaks of dark blood." Deaths usually occur on the fifth, sixth, and seventh days after infection.

The following description of the life cycle is based on the work of Clarkson (1959a). Both asexual and sexual generations occur in the upper small intestine with some spread of the latter into the lower intestinal tract. All stages, except the first generation schizont, develop both above and below the host cell nucleus with the majority occurring above. The sporozoite invades the tip of the villus and, like the sporozoite of *E. necatrix*, passes through the lamina propria into the base of a cell in the glandular epithelium where it grows into the first generation schizont containing 80–100 merozoites. These merozoites invade adjacent epithelial cells and develop into second generation schizonts with 8–16 merozoites. Third generation schizonts are somewhat similar to second but are restricted to villus epithelium; and

merozoites formed within them penetrate surface epithelium particularly at the tips of the villi, where they develop into gametocytes. According to Tyzzer (1929) and Hawkins (1952) the prepatent period is six days whereas Clarkson (1959a) reported it to be 114–118 hours.

Gill (1954) reported success in infecting week-old chickens with this species of turkey origin, but confirmation would seem to be required.

Eimeria dispersa Tyzzer, 1929, of quail origin was transmitted to three turkeys by Tyzzer (1929). The second transfer, however, did not succeed, suggesting an imperfect adaptation to the turkey, but the transfer of the same organism from the turkey back to the quail was successful. Hawkins (1952) found numerous infections in turkeys and he succeeded in transmitting it from the turkey to bobwhite and Hungarian partridges *(Perdix perdix)*. Moore and Brown (1952) also accomplished infection of both quail and turkeys with this species, but, according to Moore (1954), they were unable to infect pheasants with it.

According to Hawkins (1952) the parasites develop mainly in the epithelial cells of the villi of the upper part of the small intestine of turkeys, superficially to the nucleus of the host cell. The main gross lesions are dilation of the small intestine and creamy, sticky mucoid material in the lumen of its anterior half. The tips of the villi show necrosis on the fourth day. No marked clinical symptoms are noted except the tendency to produce liquid feces and slight depression in weight gains.

Eimeria gallopavonis was first mentioned by Hawkins (1950a) as a parasite of turkeys and the Hungarian partridge. The first published description of the species, however, was by Moore and Brown (1951) (see Table 37.2), who attributed its name to Hawkins (1950a). As used by Hawkins, however, *gallopavonis* is a *nomen nudum*. Hawkins (1952) published a complete description and stated that further study would be required to determine its pathogenicity. According to Farr *et al.* (1961) and Wehr *et al.* (1962) experimental

TABLE 37-2

Characteristics of Eimeria Affecting Turkeys*

Species	Hosts	Site of Infection	Measurements in Microns	Sporulation Time	Prepatent Period	Refractile Body	Pathogenicity
E. meleagrimitis Tyzzer (1929)	Turkey	Jejunum, Tips of villi affected (Mainly jejunum, but upper small intestine ("Glands as well as villi")	15.8–26.9 × 13.1–21.9 Av 19.1 X 16.2 (20.1 = 19.5 × 17.3 = 17) Ovad	48 hr	6 days (116 hr)	Yes	Pathogenic
E. dispersa Tyzzer (1929)	Quail Turkey Hungarian partridge Pheasant	Duodenum, also small intestine and ceca near bifurcation	21.8–31.1 × 17.7–23.9 Av 26.9 X 21.0 Broadly ovoid	48 hr	6 days	No	Relatively nonpathogenic
E. meleagridis Tyzzer (1927)	Turkey	Ceca Tips of villi involved (1st stage 15.4–20.6 remainder ceca, deep glands as well as villi)	20.3–30.8 × 15.4–20.6 Av 24.4 X 18.1 (18.2–28.1 × 13.0–20.7 Av 22.5 X 16.2) Ellipsoidal	24 hr	5 days (110 hr)	Yes	Nonpathogenic
E. gallopavonis Hawkins (1950)	Turkey Hungarian partridge	Ileum and rectum Schizonts not numerous in ileum and ceca. Residual mass present Tips of villi affected	22.2–32.7 × 15.2–19.4 Av 27.1 X 17.2 Ellipsoidal	24 hr	6 days	Yes	Pathogenic
E. adenoeides Moore & Brown (1951)	Turkey	Lower ileum, ceca, rectum. Deep glands as well as tips affected	18.9–30.2 × 12.6–20.9 Av 25.6 X 16.5 (21.5–30 × 13.5–19.5) Ellipsoidal	24 hr.	112 hr. approx. (114–132 hr.)	Yes	Pathogenic
E. innocua Moore & Brown (1952)	Turkey	Villi of small intestine	18.57–25.86 L. 17.34–24.54 W. 22.41 × 20.86 Subspherical	48 hr.	114 hr. approx.	No	Nonpathogenic
E. subrotunda Moore, Brown, and Carter (1954)	Turkey	Duodenum, Jejunum, and upper ileum. Tips of villi	16.48–26.42 L. 14.21–24.44 W. 21.77 × 19.81 Subspherical	48 hr.	95 hr.	No	Nonpathogenic

* Modified from E. N. Moore, J. A. Brown, R. D. Carter, Ohio Agr. Exper. Sta. Bars in parentheses from Clarkson (1958, 1959 a and b).

infections of *E. gallopavonis* were highly pathogenic for 3- to 6-week-old poults and caused marked symptoms and some mortality in 11-week-old poults. Except for a reduction in feed consumption and slight weight losses among heavily infected birds on the fifth day after infection the poults usually appeared normal until the sixth day when they ate little or nothing, became somnolent, and discharged fluid, blood-tinged droppings. Many poults lost weight and a few died on the sixth day. Death occurred in greatest numbers on the seventh, eighth, and ninth days and continued in diminishing numbers through the twelfth day. In severe infections the entire mucosa of the lower small intestine, the rectum, and the proximal portions of the ceca contained marked inflammatory changes. On the seventh and eighth days a milky-white exudate, composed principally of oocysts, was present throughout these areas.

According to Hawkins (1952) development occurs principally in the epithelial cells of the rectum, and, to a lesser extent, of the ileum and ceca. Marked edema, sloughing, and lymphocytic infiltration were noted in sections of the affected tissues.

Transmission of this species from the turkey to week-old chickens is claimed by Gill (1954), but it requires confirmation.

Eimeria adenoeides Moore and Brown, 1951, causes a severe enteritis in the lower small intestine, ceca, and large intestine resulting in up to 100 per cent mortality in young poults and diarrhea and reduced weight gains in poults 9 to 11 weeks of age (Clarkson and Gentles, 1958; Clarkson, 1958). According to Moore and Brown (1951), "there were no marked differences in gross pathology to distinguish this species from others affecting turkeys, other than the site of the infection." The affected regions were dilated, slightly edematous, and whitish in color owing to the presence of a caseous exudate containing enormous numbers of oocysts.

Symptoms have been described by Moore and Brown (1951), Clarkson and Gentles

(1958), and Clarkson (1958). The birds behave normally until the fourth day after infection when they eat less food and huddle together. Their feces become fluid and contain mucus and small amounts of blood. On the sixth and seventh days the feces are composed almost entirely of yellow cheesy casts and subsequently become normal. Deaths occur late on the fifth and continue to the seventh day or a little later.

In his description of the life cycle Clarkson (1958) stated that the first asexual stage is localized mainly in the terminal inch of the small intestine and "neck" of the ceca whereas later stages tend to spread into adjacent regions of the lower intestinal tract. The sporozoites penetrate epithelium on the tips or less often on the sides of the villi, rarely in the glands. They undergo nuclear division and grow into large first generation schizonts containing about 700 small merozoites. These merozoites invade both surface and glandular epithelium and develop into small second generation schizonts with 12–24 large merozoites. Generation two merozoites penetrate epithelial cells on the sides and tips of the villi and in the glands where they grow into gametocytes. Moore and Brown (1951) reported that the prepatent period was about 112 hours whereas Clarkson (1958) stated that it ranged between 114–132 hours.

Eimeria innocua Moore and Brown, 1952, seems to be appropriately named, because massive infections produced no macroscopic lesions and no signs of illness. Moore and Brown (1952) mentioned that preliminary trials suggested a slight retardation in weight gain during the first two weeks of infection. Neither chickens nor quail have proved to be susceptible to infection with this species.

Eimeria subrotunda Moore, Brown, and Carter, 1954, becomes established in the upper portion of the digestive tract to within two inches of the yolk stalk. The epithelial cells most heavily parasitized were those near the tips of the villi. Young poults infected with massive doses of

oocysts failed to exhibit symptoms or lesions. It was established that chicks, guinea fowl, ringnecked pheasants, and bobwhite quail are not susceptible to this species.

Svanbaev (1955) found unsporulated oocysts and an occasional oocyst containing two sporoblasts in feces of young turkey poults in Kazakhstan S.S.R. The oocysts were round or short oval, 21.6μ–32.8μ by 24.6μ–32.8μ, with a mean of 30.5μ by 29.8μ, and a shape index of .98. The double-contoured, smooth, greenish oocyst wall was 1.5μ to 1.7μ thick. Sporulation was completed in 16–20 hours at 20° to 25° C. A polar granule was present. The two sporocysts, 14.9μ by 10.1μ, were rounded at one end and pointed at the other and each contained 4 oval sporozoites, 7.2μ–9.0μ by 4.5μ–5.4μ. Oocystic and sporocystic residual bodies were absent. The species was named *Isospora heissini*. Svanbaev (1955) did not mention any attempt to infect other turkeys with this organism.

Slavin (1955) described and provisionally named *Cryptosporidium meleagridis*, a coccidium parasitic on the "villus epithelium" of the terminal third of the small intestine of young turkey poults from a farm in Scotland. All stages in the life cycle, except sporulated oocysts and liberated sporozoites, were observed. The oocysts were oval, 4.5μ by 4.0μ, with an eccentric nucleus. Slavin considered that the parasite was responsible for diarrhea and low mortality in 10- to 14-day-old poults.

Treatment. As testing of anticoccidial drugs proceeds, it is becoming more and more apparent that the sulfonamide drugs which are effective in chickens affect also the coccidia of turkeys (Moore, 1949; Morehouse, 1949b; Peterson, 1919; Boyer and Brown, 1953; Cuckler et al., 1956; Horton-Smith and Long, 1959). For a concise discussion of investigations on treatment of turkey coccidiosis through 1959, see Levine (1961).

Cuckler et al. (1961) reported that prophylactic feeding of .003–.025 per cent amprolium (1-[4-amino-2-n-propyl-5-pyrimidinylmethyl]-2-picolinium chloride hydrochloride) afforded complete protection from coccidiosis mortality due to E. adenoeides, E. gallopavonis, and E. meleagrimitis. Ball and Warren (1963) demonstrated that either amprolium or sulfaquinoxaline at .0125 per cent in the feed for one day before and fourteen days after infection with either E. adenoeides or E. meleagrimitis prevented much of the weight loss and all mortality. However, poults previously protected with amprolium were less resistant to severe challenge infection than were those which had received sulfaquinoxaline. These workers suggested that the two compounds differed in their action on stages in the life cycle of both E. adenoeides and E. meleagrimitis.

Horton-Smith and Long (1961) found that sulfaquinoxaline and, to a lesser extent, sulfadimidine inhibit the development of the three schizont generations of E. meleagrimitis, but have little effect on the gametocytes. They stated that the mechanism by which sulfaquinoxaline inhibits schizogony is probably associated with the PABA-folic acid sequence.

REFERENCES

Ball, S. J., and Warren, E. W. 1963. The effect of sulphaquinoxaline and amprolium against *Eimeria adenoeides* and *E. meleagrimitis* in turkeys. Res. Vet. Sci. 4:39.

Becker, E. R. 1934. Coccidia and Coccidiosis of Domesticated, Game, and Laboratory Animals and of Man. The Iowa State University Press, Ames, Iowa

Boyer, C. I., and Brown, J. A. 1953. The comparative coccidiostatic activity of some drugs against turkey coccidia. Proc. Am. Vet. Med. Assoc. 90th Meeting. P. 328.

Clarkson, M. J. 1958. Life history and pathogenicity of *Eimeria adenoeides* Moore and Brown, 1951, in the turkey poult. Parasit. 48:70.

———. 1959a. The life history and pathogenicity of *Eimeria meleagrimitis* Tyzzer, 1929, in the turkey poult. Parasit. 49:70.

———. 1959b. The life history and pathogenicity of *Eimeria meleagridis* Tyzzer, 1927, in the turkey poult. Parasit. 49:519.

———, and Gentles, M. A. 1958. Coccidiosis in turkeys. Vet. Rec. 70:211.

Cuckler, A. C., Malanga, C. M., and Ott, W. H.: 1956. The antiparasitic activity of nicarbazin. Poultry Sci. 35.98.

———, Cobb, W. R., McManus, E. C., and Ott, W. H.: 1961. Amprolium. 6. Efficacy for turkey coccidiosis. Poultry Sci. 40:1392.

Farr, M. M., Wehr, E. E., and Shalkop, W. T.: 1961. Pathogenicity of *Eimeria gallopavonis*. Va. Jour. Sci. 12:150.

Gill, B. S.: 1954. Transmissibility of turkey coccidia (*Eimeria meleagridis, E. meleagrimitis* and *E. gallopavonis*) to chickens. Indian Vet. Jour. 31:172.

Hawkins, P. A.: 1949. *Eimeria meleagrimitis* Tyzzer, 1929 in the turkey. Jour. Parasit 35 (Suppl.) :21.

———: 1950a. Coccidiosis of the turkey. Jour. Parasit. 36 (Suppl.) 42

———: 1950b. Intestinal protozoa of turkeys. Proc 53rd Ann Meet U S Livestock Sanit. Assn Columbus, Ohio, 1949. P. 114.

———: 1952. Coccidiosis in turkeys. Mich. St. Coll Agr. Exper Sta , Tech Bul No 226

Hinshaw, W. R.: 1937. Diseases of turkeys. Calif. Agr. Exper. Sta., Bul. 613.

Horton-Smith, C., and Long, P. L.: 1959 The anticoccidial activity of glycarbylamide. Brit. Vet. Jour. 115:55.

———, and Long, P. L.: 1961. Effect of sulfonamide medication on the life cycle of *Eimeria meleagrimitis* in turkeys. Exper. Parasit 11-93.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man Burgess Publishing Co , Minneapolis, Minn, 412 pp.

Moore, E. N.: 1949. Sulfaquinoxaline as a treatment for coccidiosis in turkeys. Cornell Vet 39:223.

———: 1954. Species of coccidia affecting turkeys. Proc. 91st Ann. Meet. Am. Vet. Med. Assn Seattle. P. 300.

———, and Brown, J. A.: 1951. A new coccidium pathogenic for turkeys, *Eimeria adenoeides* n sp. (Protozoa: Eimeriidae). Cornell Vet. 41:124.

———, and Brown, J. A.: 1952. A new coccidium of turkeys, *Eimeria innocua* n. sp. (Protozoa-Eimeriidae) Cornell Vet. 42:395.

———, Brown, J. A, and Carter, R. D.: 1954. A new coccidium of turkeys, *Eimeria subrotunda* n. sp. (Protozoa: Eimeriidae). Poultry Sci. 33:925

Morehouse, N. F.: 1949a. Coccidiosis as a disease of turkeys. Ann N.Y. Acad. Sci. 52:501.

———: 1949b. Experimental chemotherapy of coccidiosis in turkeys. Ann. N.Y. Acad. Sci 52:589.

Peterson, E. H.: 1949. Sulfonamides in the control of experimental coccidiosis in the turkey. Vet. Med. 44:126.

Slavin, D.: 1955. *Cryptosporidium meleagridis* (sp. nov.). Jour. Comp. Path. and Therap. 65:262.

Steward, J. S.: 1947. Host-parasite specificity in coccidia: Infecuon of the chicken with the turkey coccidium, *Eimeria meleagridis*. Parasit 38 157.

Svanbaev, S. K.: 1955. [A new species of coccidia in turkeys] Trudy Inst. Zool. Akad. Nauk. Kazakh. SSR 3:161.

Tyzzer, E. E.: 1929. Coccidiosis in gallinaceous birds. Am. Jour. Hyg. 10:269.

Wehr, E. E., Farr, M. M., and Shalkop, W. T.: 1962. Studies on pathogenicity of *Eimeria gallopavonis* to turkeys. Jour. Protozool. 9(suppl.):8.

COCCIDIOSIS OF THE GOOSE

More is known concerning coccidiosis of geese than of ducks, but our information is still meager, particularly regarding the intestinal forms. The goose alone of domestic poultry is afflicted with renal coccidiosis caused by *Eimeria truncata*. The intestinal species *E. anseris* and *E. nocens* are rarely found, but may be pathogenic in severe infections. The three species described by Farr (1953) from the Canada goose (*Branta canadensis*) which she was able to transfer successfully to the common goose—*E. hermani, E. striata,* and *E. fulva* —will not be further discussed here.

Eimeria truncata Railliet and Lucet, 1891, a highly pathogenic parasite of young geese, causes destruction of kidney tissue, particularly the epithelium of the uriniferous tubules. The disease is very acute, lasting but 2 or 3 days, and is almost always fatal. Usually the mortality in a flock is very high. The clinical signs are extreme weakness and emaciation. The kidneys are enlarged and studded with poorly circumscribed, small, yellowish-white streaks and spots. The tubules are dilatated by masses of oocysts and urates.

Opinions differ as to the morphology of this species. The oocysts, $11.7\mu-21.6\mu$ by $14.4\mu-27\mu$, are ovoid, elliptical, or round with one end drawn out a little and sharply cut off. At the truncated pole is a more or less prominent micropyle surmounted,

in some cases, by a membranous cap. The oocyst wall is smooth, colorless, and delicate. At the completion of sporulation, which requires 1–5 days, an oocystie residual body is sometimes found among the sporocysts. According to Lerche (1925) the sporocysts, 7.8μ by 5.5μ, contain two comma-shaped sporozoites and a finely granular sporocytic residuum. (See also McGregor, 1952; Ridala, 1936; and Spiegl, 1921.)

This disease was known only in Europe until McNutt (1929) reported an outbreak in Iowa. The reports and reviews by Levine *et al.* (1950) and Lindquist *et al.* (1951) indicate the prevalence of this parasite in Illinois, Maryland, Michigan, District of Columbia, New York, Washington, Quebec, and Ontario.

E. truncata was reported from goslings of the graylag (*Anser cinereus*) by Christiansen and Madsen (1948) and Christiansen (1952). The last-named author also found in the kidneys of young swans (*Cygnus olor*) oocysts resembling *E. truncata*, but smaller. He also found in the kidneys of common eiders (*Somateria mollissima*) oocysts of a small variant of *E. truncata*. Waldén (1965), believing that the small form from kidneys of *Cygnus olor* was not *E. truncata*, gave it the name *E. christianseni*. He also stated that Christiansen's small form of *E. truncata* from eiders was probably specifically distinct. Pavlov (1942) seems to have found *E. truncata* in the kidney of the domestic duck.

The following descriptions of goose coccidia are based mainly on the work of Kotlán (1933). (See also Cerná, 1956; and Hanson, Levine, and Ivens, 1957.) *Eimeria parvula* Kotlán, 1933, a common harmless parasite was found chiefly in the epithelium of the terminal small intestine. The round or round-elliptical oocysts, 10μ–15μ by 10μ–14μ, had a colorless, delicate wall without a micropyle.

Eimeria nocens Kotlán, 1933 developed within and under the epithelium of the tips of the villi in the posterior small intestine. The brownish oocysts, 25μ–33μ by 17μ–24μ, were egg-shaped with a more or less truncated pole containing a micropyle. *E. nocens* was an uncommon parasite, found principally in young geese with intestinal disturbances. Kotlán (1933) believed that further investigations would be required to determine its pathogenicity.

Stages in the life cycle of *E. anseris* Kotlán, 1932 occurred under and within the epithelium of the posterior small intestine. Experimental infection was apparently harmless for 2½- to 3-month-old geese. The colorless oocysts, 16μ–23μ by 13μ–18μ, were pear-shaped with an evident micropyle at the tapered pole. According to Hanson *et al.* (1957) *E. anseris* oocysts from *Anser c. caerulescens* measured 16μ–19μ by 20μ–21μ. The smooth colorless wall of the oocyst was sharply incised at the narrow pole to form a plate across the micropyle. Below the plate was an amorphous oocystic residuum. The sporocysts, 7μ–9μ by 10μ–12μ, were ovoid in shape and contained two sporozoites and a large residuum. Bajard (1962) stated that symptoms of intestinal coccidiosis due (presumably) to *E. anseris* and *E. nocens* were anorexia and constipation followed by diarrhea. In severe cases death came quickly. Otherwise the geese improved after two or three days of diarrhea.

Tyzzeria anseris Nieschulz, 1947. The description of this species was based on the morphology of oocysts found in feces of a domestic goose in Netherlands. The elliptical oocysts, 12.0μ–16.0μ by 10.0μ–12.5μ, had a thick colorless wall without a micropyle. Upon sporulation they contained 8 free sporozoites and an eccentric oocystic residual body. Nieschulz could not infect a duck with *Tyzzeria anseris*. For a discussion of the problem of the identity of *T. anseris* and of other species of *Tyzzeria* reported from ducks and wild geese and swans see Hanson *et al.* (1957); and Levine (1961). Farr and Wehr (1952) found the same species in two goslings from Maryland.

REFERENCES

Bajard, M.: 1962. Maladies parasitaires du tractus digestif de l'oie dans les Landes Alfort École Nat. Vet. These (46), 62 pp.

Černá, Ž.: 1956. [Contribution to the knowledge of the coccidia of geese.] Věstnik Československ. Zool. Společ. Praze 20:366.

Christiansen, M.: 1952. Nyrecoccidiose hos vildtlevende andefugle (Anseriformes). *Eimeria somateriae* n. sp. hos ederfugl (*Somateria mollissima* L.). Nordisk Vet.-med. 4:1173.

—— and Madsen, H.: 1948. *Eimeria bucephalae* n. sp. (Coccidia) pathogenic in goldeneye (*Bucephala clangula* L.) in Denmark. Danish Rev. of Game Biol. 1:61.

Farr, M. M.: 1953. Three new species of coccidia from the Canada goose, *Branta canadensis* (Linné, 1758). Jour. Wash. Acad. Sci. 43:336.

——, and Wehr, E. E.: 1952. *Eimeria truncata* associated with morbidity and death of domestic goslings. Cornell Vet. 42:85.

Hanson, H. C., Levine, N. D., and Ivens, V.: 1957. Coccidia (Protozoa: Eimeriidae) of North American wild geese and swans. Canad. Jour. Zool. 35:715.

Kotlán, A.: 1933. Zur Kenntnis der Kokzidiose des Wassergeflugels Die Kokzidiose der Hausgans. Zentralbl. f. Bakt., I. Orig. 129:11.

Lerche, M.: 1923. Nierencoccidiose bei Hausgänsen. Zeitschr Infektionskr Haustiere. 25:122.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and Man. Burgess Publishing Company, Minneapolis, Minn. 412 pp.

——, Morrill, C. C., Schuttle, S. C.: 1950. Renal coccidiosis in an Illinois gosling. No. Am. Vet. 31:738.

Lindquist, W. D., Belding, R. C., and Hitchcock, D. J.: 1951. A report on the presence of renal coccidiosis in Michigan. Mich. St. Coll. Vet. 12:19

McGregor, J. K.: 1952. Renal coccidiosis in geese. Jour. Am. Vet. Med. Assn. 121:452.

McNutt, S. H.: 1929. Renal coccidiosis of geese. Jour. Am. Vet. Med. Assn. 75 565

Nieschulz, O.: 1947. Eine neue Kokzidienart bei der Hausgans. Zentralbl. f. Bakt I Abt. Orig. 152:74.

Pavlov, P.: 1942. Coccidienbefunde bei Saugetieren und Vogeln in Bulgarien. Zentralbl. f. Bakt. I, Abt. Orig. 149:317.

Rídala, V.: 1936. Hanede neerukoktsidioos [Renal coccidiosis of geese]. Eesti Loomaarstlik Ringvaade. 12:177.

Spiegl, A.: 1921. Nieren-Kokzidiose bei Hausgänsen. Zeitschr. Infektionskr. Haustiere 22:265.

Waldén, H. W.: 1961. Observations on renal coccidia in Swedish anseriform birds, with notes concerning two new species, *Eimeria boschadis*, and *Eimeria christianseni* (Sporozoa, Telosporidia). Arkiv Zool. 15:97.

COCCIDIOSIS OF DUCKS

A number of accounts of losses in domestic ducks have been attributed to coccidia, but the etiology of these cases has not always been sufficiently elucidated. (See Dougherty, 1952, 1960; Davies, 1957.)

Tiboldy (1933) described oocysts of the genus Eimeria that were oval, elongate oval, or occasionally rounded, possessed a sturdy wall, and measured from 10.8μ–25.0μ by 8μ–12.6μ. A number of species are possibly involved, but the question of specificity has not been cleared up. Scholtyseck (1955) has described *E. anatis* from the European wild duck, *Anas platyrhynchos*, but did not find it in the domesticated varieties. The wall of the ovoid oocyst is smooth, homogeneous, and 0.7μ–1.0μ thick. There is a ringlike thickening about the more pointed end and, after sporulation,

a characteristic roundish thickening on the inside of the wall enclosed by the ring. Residual granules are present among the sporozoites. The oocysts measure 14.4μ–19.2μ by 10.8μ–15.6μ; mean, 16.8μ by 14.1μ. Schizonts and gametocytes were observed in the small intestine. Pavlov (1942) found *E. truncata*, more commonly occurring in geese, in the kidneys of domestic ducks in Bulgaria.

The only other described species from domestic ducks is *Tyzzeria perniciosa* Allen, 1936, from the small intestine of its host. It was described from a six-week-old Pekin duck (*Anas domesticus*) obtained from Rinebeck, New York, U.S.A. The oocysts of this genus are peculiar in that they are asporocystic (without sporocysts) and after development for 24 hours outside the body contain eight sporozoites

and a large residual mass. The content of the freshly passed oocyst, however, consists of coarsely granular protoplasm completely filling the oocyst. The asexual developing stages appear in the mucosa of the small intestine from the gizzard to the ceca. There are three morphologically distinguishable generations of schizonts after which (about 48 hours after inoculation) gametocytes make their appearance. Oocysts were first observed in the tissue by the end of the fifth day and in the droppings on the sixth day.

The species is extremely pathogenic, for Allen lost seven of ten very young ducks which she experimentally infected. The symptoms reported as "loss of appetite and weight, weakness manifested by the inability of the bird to stand for any length of time, and continuous crying as if in distress. The last symptom was especially noticeable in baby ducks."

Macroscopic lesions consisted of inflammatory and hemorrhagic areas throughout the small intestine, especially in the upper half. The intestinal wall was thickened and exhibited rounded white spots on the exterior. In severe cases blood and cheesy exudate, but no core, filled the small intestine.

Tissue sections revealed the penetration of the coccidium into the mucosa and submucosa as far as the muscular layers, and extensive tissue destruction. The possible relationship of *Tyzzeria* species occurring in various ducks and geese is discussed in the previous section. See also Levine (1953).

REFERENCES

Allen, E A. 1936 *Tyzzeria perniciosa* gen. et sp. nov., a coccidium from the small intestine of the Pekin duck, *Anas domesticus* L. Arch. f. Protistenk. 87:262.

Davies, S F. M. 1957. An outbreak of duck coccidiosis in Britain. Vet. Rec. 69:1051.

Dougherty, E. 1952. Coccidiosis in ducks. Rep. N.Y. State Vet. Coll. (1950–51), p. 31.

———. 1960. Duck coccidiosis. Rep. N.Y. State Vet. Coll. (1958–59), p. 48.

Levine, N. D: 1953. A review of the coccidia from the avian orders Galliformes, Anseriformes and Charadriiformes, with descriptions of three new species. Am. Midland Naturalist 49 696.

Pavlov, P.. 1942. Coccidienbefunde bei Säugetieren und Vögeln in Bulgarien. Zentralbl. f. Bakt. I. Abt. Orig 149 317

Scholtyseck, E.: 1955. *Eimeria anatis* n. sp. ein neues Coccid aus der Stockente (*Anas platyrhynchos*). Arch. f. Protistenk. 100.431.

Tiboldy, B.: 1953. Experimentelle Untersuchungen über die Spezifität der Kokzidien des Hausgeflügels. Extract of thesis, Budapest.

ENTEROHEPATITIS (BLACKHEAD)[2]

Some phases of blackhead are dealt with in the chapter on diseases of the turkey. This section is concerned mostly with the etiological agent, *Histomonas meleagridis*, and other organisms associated with its transmission.

Histomonas meleagridis (Smith, 1895) Tyzzer, 1920

In 1895 Theobald Smith identified a protozoan, which he called "*Amoeba meleagridis*," as the cause of blackhead. He described from sections of lesions spherical or slightly oval bodies from 6μ–10μ in diameter, with a small spherical nucleus 2μ in diameter, in some cases with a nucleolus. In the original paper Smith did state

that movements characterized as ameboid had not yet been demonstrated, but in 1910 Smith again studied the fresh microorganisms from the liver (which were from 8μ–15μ in diameter) in a warm chamber and noted slight changes of form (Fig. 37.5). He states: "Some of the freed parasites . . . pushed out small finger-like pseudopodia, usually one at a time." The arrangement of the organisms singly or in groups in the tissues and unenclosed by a common membrane suggested that multiplication took place as a simple process of division and not as endogenous segmentation.

Tyzzer (1919) first noted adjacent to the nucleus of the protozoan an "extranuclear body" from which delicate filaments radiated over the surface of the nuclear membrane. Division of the extranuclear body
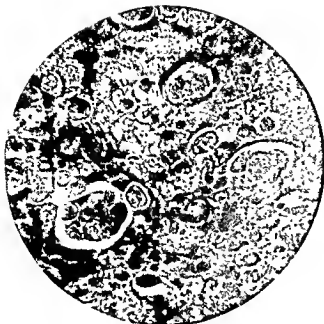
FIG. 37.5 — Liver from turkey affected with blackhead showing the characteristic parasites. ×920. (Biester, Iowa State University.)

resulted in the formation of a paradesmose which persisted until division of the nucleus had taken place, after which cell division occurred. No flagella had been observed at this time, but amoeboid movement had been encountered frequently. Nevertheless, Tyzzer remarked that "the presence of an extranuclear body resembling the blephroplast of flagellates and a type of nuclear division characteristic of certain flagellates, sets it apart from all known amoebae." But he later concluded "reclassification of the parasite should not be attempted until additional facts have been obtained concerning its life history."

Many of the "additional facts" concerning the life history of the protozoan were slow in coming, but in less than a year Tyzzer (1920b) had succeeded in inducing the organisms to move briskly, and in a variety of ways. One type of movement was so suggestive of flagellar activity that he renamed the organism Histomonas meleagridis, even though the much sought external flagella had not yet been observed.

Free flagellated forms were first seen by Tyzzer and Fabyan (1922b). These were taken from the ceca of experimentally infected turkeys. They state that late in the disease they found in the ceca of turkeys forms of Histomonas meleagridis in a considerable proportion of which one or two short flagella were demonstrable. In 1924 Tyzzer found flagellate forms in the ceca of chickens. Drbohlav (1924) obtained the flagellated forms in cultures from the ceca of diseased birds. Flagellate types show a great variety of ameboid movement and ingest bacteria, cell fragments, and starch grains (Tyzzer, 1924).

Tyzzer (1934) later gave us more light on the nature and behavior of Histomonas. In cecal discharges under optimum conditions it is fairly rounded, but with irregular surface extensions, and exhibits active ameboid movements and rhythmic rotary movements. At the proper high temperature the rhythmic beat becomes frequent, and a flagellum may be seen beating in one direction toward the body, causing it to rotate one-fourth to one-third of a full rotation at each stroke. One form was seen with four flagella.

In cultures the organism usually attains a larger size than in cecal discharges. At times culture forms are spread out into thin sheets and exhibit active ameboid movements; at other times they are more rounded and undergo "rhythmic flagellate motility." When brought into contact with

surfaces, the latter forms may become ameboid while still exercising their flagella. The flagellated phases may measure from 4.5μ–5μ in diameter, and flattened ameboid forms considerably more. The cytoplasm may contain starch and bacteria in considerable quantities. While normally uniflagellate, aflagellate forms are common, and those with two or even four flagella are not infrequently encountered in cultures.

DeVolt and Davis (1936) have confirmed in large part Tyzzer's observations on the behavior and nature of the organism from tissues and in cecal discharges and culture.

Lund (1963) described as a new species a histomonad that does not produce blackhead in either chickens or turkeys, but is transmitted in the same ways as is *Histomonas meleagridis*. This organism, named *H. wenrichi*, inhabits the lumen of the cecum, and apparently does not invade the tissues, nor multiply appreciably therein, if liberated in the mucosa by cecal worm larvae. Its dimensions are about 1.5 times those of *H. meleagridis*. It characteristically has four flagella and it feeds primarily on bacteria. It has thus far failed to grow on any of the artificial media used to cultivate *H. meleagridis*, and cannot, therefore, be likened to the large histomonads occasionally seen in cultures of *H. meleagridis*. Neither can its lack of pathogenicity be attributed to prolonged growth on artificial media (see section on "Immunity and Immunization"). Tyzzer undoubtedly had some of these large, nonpathogenic histomonads mixed with *H. meleagridis* in some of his chickens, and he published the first figures of it (1934, Figs. 26 and 28; also possibly, 1927, Fig. 3). Wenrich (1943) found the organism, apparently in the absence of *H. meleagridis*, in two pheasants, and he described it in considerable detail. However, he did not attempt transmission or cultivation, and concluded that the organism was a variant of *H. meleagridis*. *H. wenrichi* is apparently quite common in chickens, turkeys, and

pheasants, but it thrives best in birds not harboring the more prolific *H. meleagridis*. The diagnostician should distinguish it from the pathogen to avoid recommending the institution of expensive and needless control measures for flocks in which blackhead is not present. To the researcher, the use of the nonpathogenic species may have advantages for certain types of studies (Lund and Burtner, 1957; Lund 1959, 1961).

Transmission in Relation to Life History

Blackhead has been produced experimentally in a variety of ways with varying degrees of success. The results of more than two dozen such efforts have been tabulated by Swales (1950 a and b). Evidence of transmission through the egg of the turkey or chicken is entirely lacking. Consequently, infection as a result of ingestion of Histomonas, alone or with other organisms, remains the only plausible means of acquisition of the protozoon under natural conditions.

1. Direct infection. Smith (1895) assumed that "the microparasite is transmitted from bird to bird" without the intervention of an intermediate host. Moore (1896) appeared to have demonstrated this possibility by feeding viscera and droppings from infected birds. However, most of the early workers had difficulty finding the organism in the discharges from infected turkeys (Tyzzer, 1919, 1920a, and Tyzzer and Fabyan, 1920). Tyzzer (1924) found them in relative abundance in the cecal contents of chickens, and Curtice (1907 a and b) had shown that soil traversed by chickens was particularly hazardous as a source of blackhead infection. It is, of course, now known that turkeys also void histomonads in their cecal droppings at times. But the histomonad was known to be a fragile organism, capable of living only a few hours in cecal discharges (Tyzzer and Collier, 1925, and others), and no cyst or comparably resistant stage could be found. This made it difficult for the early workers to explain

how birds were acquiring blackhead by contact with soil or quarters long unused by poultry. As we shall see presently, the protozoan actually does have other means of dissemination, accompanied by adequate protection.

Nevertheless, direct infection without the intervention of other organisms had occasionally been demonstrated experimentally, but evidence to support the reasons postulated for the many failures was lacking. Horton-Smith and Long (1955, 1956a) found that by raising the pH of the gizzard of chickens from its normal low of 2.9–3.3 to 6.7–7.0 by starvation, or to 7.3–7.6 by giving alkaline solutions, the birds could be infected by oral administration of histomonads in cecal droppings. About the same time Lund (1956) found that turkey poults could be more readily infected with histomonads given orally by using inocula containing very little solid material than by using ones containing material subject to digestion, thereby encouraging retention in the gizzard. Both studies pointed to the same circumstance—the unprotected histomonads could not survive long exposure to the acid of the gizzard. It now became clear that natural infection by the ingestion of Histomonas alone was possible, but that it was unlikely among birds with ready access to clean water and feed. Water that had been grossly contaminated with cecal droppings shortly before a hungry bird came to drink might still constitute a hazard for that bird.

2. The relationship of Heterakis gallinarum to blackhead. The initial demonstration of the role of Heterakis, the nematode worm commonly inhabiting the ceca of chickens, turkeys, and other fowl, was made by Graybill and Smith (1920). First two turkeys were fed feces of adult turkeys and cultures of embryonated Heterakis ova (from chickens) kept in Petri dishes in normal saline solution for 17 days. Both infected turkeys became sick 15 days after ingesting the ova, and both died within a week. Three more turkeys given the same feed

contracted blackhead, as did three which received only the embryonated eggs. Negative results were obtained from feeding turkeys the feces only.

It is apparent that Graybill and Smith were of the opinion that the cecal worms, invading the ceca in large numbers, broke down the resistance of the fowl to the protozoan factor which was already present in the ceca, "probably disseminated when the first spontaneous cases occurred in the stock." They suggested that there might likewise be other agents which, when ingested by the turkeys, would prepare the way for invasion of the tissues by the blackhead organism. In the same year, however, Smith and Graybill showed that the disease could be produced in chickens by feeding incubator-hatched chicks an overdose of the eggs of Heterakis which had been placed in 0.5 per cent solution of bichloride of mercury for 30 seconds, washed, and dried. This time they concluded that the evidence pointed to the presence of Histomonas (=Amoeba) meleagridis in the cultures, but they were disinclined to accept this possibility unreservedly and so maintained that the origin of the protozoan was still undetermined. Tyzzer, Fabyan, and Foot (1921) confirmed in principle the work of Graybill and Smith, and noted likewise that it was not necessary to feed the protozoan with the embryonated eggs in order to produce the disease. Swales (1950) found that blackhead was not transmitted from carrier birds to susceptible poults in the absence of embryonated Heterakis eggs.

That the ovum of Heterakis may actually harbor Histomonas inside its shell has received very strong support from the work of Tyzzer and Fabyan (1922b). Heterakis material treated for 3 days with 1.5 per cent nitric acid, which rendered the medium bacteriologically sterile, was proved capable of transmitting blackhead when fed to incubator-bred turkeys. The discharges of blackhead carriers free from embryonated eggs did not transmit the disease after treatment with 1.5 per cent

nitric acid (Tyzzer, 1926). Swales (1948) showed that the histomonad was present within infective larvae separated from embryonated eggs of Heterakis.

Lund and Burtner (1957), working with a nonpathogenic histomonad, found that fewer than one out of 200 of the worm eggs used in their studies transmitted the protozoan, but recognized that this ratio did not necessarily pertain to pathogenic histomonads. Kendall (1957) observed that it seemed "necessary to give at least 1,000 embryonated" (Heterakis) "eggs to each poult in order to set up disease." Actually, this ratio varies greatly according to the source of the worms (unpublished data from files of senior author).

Tyzzer and Fabyan showed that the disease could be produced in turkeys by feeding hen-yard soil which, presumably, contained embryonated Heterakis eggs. Swales (1948) demonstrated transmission of Histomonas in Heterakis larvae mechanically separated from Heterakis eggs.

For many years the weak link in the chain of circumstances incriminating Heterakis eggs was the failure to demonstrate the protozoan inside their membranes. Glaser (1921) was unable to find histomonads in infective cultures of Heterakis ova, and Tyzzer (1926) was likewise unable to find the organism in the ovum, although he reported that "the invasion of the tissue of the worm has been demonstrated in a number of instances, thus far in half-grown worms from cases of blackhead." He later (1927) published figures showing several histomonads in the intestinal epithelium of heterakids 11 days old.

Niimi (1937) found what he believed to be histomonads not only in the lumen and the wall of the gut of developing cecal worms, but also in the terminal portion of the ovary and uterus of mature worms, and in fertile eggs, some of which had undergone cleavage. Niimi's organisms in the gut and gut wall corresponded in form and size with those figured earlier by Tyzzer, but the bodies assumed to be histomonads in the ovary and the eggs were much

smaller, 1μ–1.4μ in diameter, with nuclei 0.5μ–0.7μ in diameter. Niimi's work may not have received the attention it deserved, for various reasons. The journal in which it appeared seems not to have a wide circulation in this country, and accurate translation of the article is very difficult. Moreover, to some it seemed incredible that an organism could be reduced to less than $\frac{1}{3}$ its usual dimensions, nucleus and all (and, presumably, thus to less than 1/100 its usual mass) by simply migrating from the worm's gut to its ovary.

Recently Gibbs (1962) demonstrated histomonads not only in the eggs of Heterakis and throughout most of the reproductive tract of the female worm, but in the lumen and walls of the gut and in the reproductive tract of male worms also. At all sites, the range in size of the histomonad was comparable to that of the protozoan in the tissues of the bird. Dividing forms were seen in the ovary and in the eggs.

Kendall (1959) figured and described four "Parasites resembling *Histomonas meleagridis*" in a 4-day Heterakis larva. Presumably, these histomonads were present in this larva as the egg containing it was ingested by the host bird. Those viewed by Tyzzer, Niimi, Gibbs and others in larvae of 11 days or more were probably for the most part acquired from the host bird as the larvae fed in the infected ceca. Such histomonads would not initiate infections in new hosts until liberated there by the next generation of worms. Obviously, some Histomonas-bearing larvae of each generation must liberate their protozoa if the avian host is to acquire the Histomonas infection, and some of the developing larvae must acquire histomonads from the bird if the larvae of the next generation are to transmit the protozoan with comparable certainty. This does not, of course, preclude the possibility of Heterakis eggs becoming carriers of the same histomonads that were brought in by larvae that gave rise to those eggs, but such a precarious arrangement would, by itself, hardly insure the continued success of the protozoan. Furthermore, both Niimi and

Lund (1961), the latter working with a nonpathogenic histomonad, have shown that Histomonas-free Heterakis can be made to produce Histomonas-bearing ova by growing both parasites in the same birds simultaneously. The nonpathogenic histomonad was apparently acquired most readily by larvae 9 to 18 days old, and most frequently liberated by larvae 6 to 14 days old. These periods are not so well established for the pathogenic histomonad, but are not identical (senior author's unpublished data). In any event, the periods of liberation and acquisition of the histomonads by the cecal worms are such that worms from a single ingestion of Histomonas-bearing Heterakis ova have reasonable assurance of producing some eggs that also carry histomonads, if the worms survive to maturity. As Tyzzer and Fabyan (1922b) and many others have recognized, an active case of blackhead in a turkey frequently makes the cecum an untenable habitat for the worms.

From the above it is apparent that the cecal worm not only plays a very intimate part in the transmission of blackhead, but that it is admirably adapted to perform its role. Its egg is a sturdy body, well suited to survival under most natural conditions likely to be encountered in environments resembling those to which its hosts are adapted. It resists short periods of desiccation, considerable periods of freezing, and remains viable over relatively long periods under more favorable conditions. Riley and James (1921) observed that "Dry cultures which had attained the coiled embryo stage remained latent for 6 weeks, and promptly became motile on the addition of moisture." They further noted that 50 per cent of the embryonated eggs held 3 days at temperatures between −10° and 0° F., followed by storage for 6 months at 0°–10° F. were capable of segmentation, and many of these completed embryonation "in practically a normal period." Graybill (1921) found that undeveloped ova "survived desiccation at room temperature for a period of 16 days, but not 41 days. Fully developed eggs were alive after

desiccation for 18 days, but not after 49 days. In another instance they were no longer viable after 10 days." He further noted that embryonated eggs kept at room temperature in physiological saline survived during a period of a little over 12 months, while those kept in soil outdoors (New Jersey) contained living embryos after a period of 8 months.

Niimi (1937) stated, "The causative agent of the disease in question [blackhead] may survive in worms' eggs longer than 1 year, perhaps 3 or 4 years." Farr, working in Maryland, (1956, 1961) found that Heterakis ova that had been in experimental plots inaccessible to chickens or turkeys for as long as 210 to 230 weeks were still capable of producing worms in poults. Histomonas was not transmitted after 151 weeks, but the number of worms recovered after feeding eggs older than 3 years was small. Lund (1960), also working in Maryland, reported that worm counts in turkey poults exposed periodically to naturally infected test plots declined 89 per cent after the plots had been vacant for 5 summer months, another 8 per cent after the passage of 7 cool months, and was down to only 0.8 per cent of the original after the second summer. After that, 75 to 96 per cent of the birds (usually 160 to 180 per test) harbored no worms. The incidence of blackhead did not decline at a corresponding rate, falling by 35 per cent, 10 per cent, and 45 per cent after the first summer, the 7 cooler months, and the second summer, respectively. Thereafter, both worm counts and the incidence of histomoniasis declined slowly to very low levels until 52 months had elapsed. After that, both parasites almost disappeared, histomonads having been observed in only one of 167 birds released at 60 months, and in none of 176 released 65 months after the naturally contaminated plot had been vacated for testing. As we shall see presently, it may be virtually impossible to rule out completely the possibility of recontamination of soil which is maintained sufficiently close to its natural state to make results meaningful.

3. The relationship of the chicken to the dissemination of blackhead among turkeys. Only a few years after Cushman (1893) had called attention to blackhead as a specific disease among turkeys, and Smith had found the protozoan responsible for it, Chester and Robin (1901) reported the presence of the disease in chickens. Curtice (1907c) concluded, "that the poultry yards are heavily infected; that chickens, though rarely affected by blackhead disease, may be the most potent factor in its dissemination, and that diseased turkeys are nearly or quite as serious a source of infection as chickens." Milks (1908), however, reported mortality as high as 30 to 50 per cent among young chicks in Louisiana.

Following these studies, blackhead was reported repeatedly in chickens (cf. Higgens, 1915; Tyzzer, 1919 and 1924; Smith and Graybill, 1920; Kaupp, 1922; Eriksen, 1925).

The course of the infection in the common fowl usually runs a much milder course than in the turkey but is otherwise very similar. Smith and Graybill found that in chicks experimentally infected with Heterakis eggs, the initial lesions appeared in the ceca, usually followed by only microscopic focal collections of lymphocytes or yellowish necrotic specks in the liver. The inflammation and thickening of the cecal wall and the subsequent formation of a core resemble somewhat the condition in the turkey disease, but the invasion of the liver lesions bears no comparison in the two birds. Smith and Graybill state that all their chicks would probably have survived had they not been killed, for the processes of repair had been initiated, whereas experimentally infected turkeys usually died.

Reports such as those by Milks, Kaupp, and Eriksen indicate, however, that at times the disease of chickens may run a much more severe course. Kaupp reported the death of 42 out of 43 Silver Spangled Hamburg chicks about five weeks of age. Eriksen necropsied a total of 25 birds from 17 Missouri flocks. The losses ranged from one bird in a flock of 350 to more than 50 per cent in two other flocks. At necropsy cecal and liver lesions were observed, the latter organ occasionally enlarged to several times its normal size and studded with gray and grayish-yellow areas 3 to 8 mm. in diameter which penetrated deeply into the liver tissue. Histomonas was observed in sections of both ceca and liver. The age of the chicks attacked was from seven to ten weeks (cf. observations of Milks). Thus the infection in chickens may assume a serious nature at times, although under ordinary circumstances it appears to be well tolerated by the host.

There are indications that blackhead losses among chickens may have increased in recent years. Ohara and Reid (1961) calculated the frequency with which histomoniasis was recorded in proportion to accessions of diagnostic laboratories in northeastern, southeastern, and midwestern states, and report that "1.2 to 5.5 percent of the chickens in diagnostic laboratories show histomoniasis. There is some evidence of slight increase in this disease in the Midwest area." Their own experiments on age of greatest susceptibility led them to conclude, "When histomoniasis was experimentally induced with eggs of Heterakis gallinae, chickens were more susceptible at 32 days than at 1 day, 46, or 64 days of age. Comparisons with field reports indicate that very young birds and birds three months of age show some natural resistance to the organism." Desowitz (1951) and Madsen (1962) have reported similar experiences. No mortality was induced in test birds used by Ohara and Reid, but blackhead occurring concurrently with cecal coccidiosis resulted in a mortality of 12.5 per cent as compared with 6.3 per cent for cecal coccidiosis alone. Stress factors of many sorts may be expected to contribute to the severity of blackhead in chickens, but interpretation of the findings is sometimes perplexing (Madsen, 1962).

The elimination of cecal worms as a result of histomoniasis in chickens is similar to that for turkeys (Lund, 1958; Ohara and Reid, 1961), but because many more chickens survive the disease than do young

poults, the early observations of Curtice regarding the importance of the chicken as a source of dissemination of the disease are still valid.

4. Other possible factors in the transmission of blackhead. Prior to the discovery of the transmission of blackhead by the cecal worm, many means of transmission were considered and tested, usually without success. Not infrequently control birds that apparently had no access to sources of infection developed blackhead. Knowing the role of the cecal worm clarified much of the confusion, but some of the early reports are still worthy of consideration.

As early as 1907, Curtice produced blackhead in turkeys by feeding earthworms from an infected turkey yard, but not by feeding such worms from clean plots. He concluded (1907b and c), "The earthworms in this instance were probably carriers of infected soil and were not necessarily a second host to the parasite." Ackert (1917) infected chickens with Heterakis gallinarum by feeding earthworms, but blackhead, if present at all, did not come to his attention. Madsen (1962) reported having transmitted Heterakis without blackhead by feeding chickens earthworms from pheasant flight cages, but on another occasion 6 of 10 chickens fed earthworms from a different pheasant farm developed blackhead. Madsen remarks, "Before being fed to the chicks, the worms were thoroughly washed and allowed to empty their intestines as much as possible. Nevertheless a certain amount of contents must have remained, as the group contracted infections with Heterakis gallinarum and blackhead." Lund et al. (1963) presented evidence that earthworms transmit Heterakis, and also blackhead, as true hosts to the cecal worm, and not merely as carriers of embryonated heterakid eggs adhering to the cuticle, or in transit in the intestine. Heterakis larvae, sometimes in considerable numbers, were found in the coelom of some worms. If such larvae can accumulate over extended periods, earthworms may be of considerable importance in the transmission of both Heterakis and Histo-

monas by serving to concentrate infective stages, and by providing a motive for the birds acquiring these stages, sometimes in considerable numbers. Consequently, absolute freedom from contamination of outside soil plots or range can hardly be assured. The earthworms migrate to some extent in the soil, and on the surface, under some conditions. Birds carrying worms to their nests may drop them, thus providing a source of contamination for plots well removed from soil contaminated by infected birds.

Arthropod transmission of blackhead has been considered by many workers. Smith (1895) dismissed the possibility because "certain flocks only are infected." Curtice (1907a) raised poults free of blackhead although "they undoubtedly ate all sorts of insects." Tyzzer and Fabyan (1920) failed to produce blackhead in a turkey fed 185 laboratory-raised blowflies (Calliphora erythrocephala) that had recently been allowed to feed on lesions from experimentally infected birds. Another turkey that ate crickets and grasshoppers in a field adjoining its pen also remained healthy. Tyzzer et al. (1921) also failed to infect any of 3 poults that were permitted to consume large numbers of blowflies that had frequented adjoining poultry yards. However, DeVolt and Davis (1936) had 5 cases of blackhead among 67 poults that consumed trapped houseflies, and Frank (1953) was able to transmit blackhead to poults by feeding houseflies, certain flesh flies (Lucila), and grasshoppers of several species.

The recent studies on the direct transmission of Histomonas in the absence of Heterakis ova indicate that transmission by the ingestion of insects with histomonads, but no heterakids, is unlikely. Presumably, any insect, or any other organism, in contact with the soil and capable of carrying Heterakis ova, externally or internally, could serve as a mechanical carrier of histomonads.

The absence of Histomonas in the peripheral circulation of birds has been taken by most investigators to indicate that

were ingested. The identity of the flagellate cultured with the forms found in blackhead was proved by rectal inoculations into chickens, which showed large numbers of the parasites in the cecal discharges within 3 days and then developed typical blackhead. Tyzzer (1934), DeVolt and Davis (1936), and Bishop (1938) also obtained very successful cultures of *Histomonas meleagridis* using various modifications of the diphasic medium employed by Drbohlav. Bishop was apparently the first to culture organisms from liver lesions. She was also the first to report the continued propagation of Histomonas on a liquid medium, employing that used by Laidlaw et al. (1928) for estimating the toxicity of emetine for *Entamoeba histolytica*. Delappe (1953a, b, c, and d), using Laidlaw's medium, found, among other things, that Histomonas is a facultative anaerobe.

DeVolt (1943) grew *Histomonas meleagridis* in a medium consisting essentially of Locke's solution with glucose reduced to 0.2 gm. per liter, to which had been added 2 per cent turkey or chicken serum, and 2 per cent N/20 NaOH to prevent the precipitation of the serum as the medium was autoclaved.

Various workers have used modifications of DeVolt's medium. Lesser (1960a) omitted the N/20 NaOH of the medium considerably, and added sterile bovine serum after autoclaving. He also showed that cream could replace the serum in the above-mentioned medium, and could also be used in a modification of tissue culture medium "199" (Lesser, 1960b). He later (1961b) reported that certain cholesterol compounds could be used instead of cream. However, the cream had the ability to protect the histomonads from the adverse effects of amphotericin B, whereas the cholesterol compounds did not (1963). Lesser in all instances used antibiotics to kill the bacteria which were added to the Histomonas cultures. He found that he could dispense with the dead bacteria if fresh hamster liver tissue was added to the medium (1961a).

Goedbloed and Bool (1962) grew *Histomonas meleagridis* of liver origin in the culture media of Dobell-Laidlaw and Boeck-Drbohlav to which were added *Escherichia coli*, *Staphylococcus aureus*, or *Klebsnella*. These authors do not specify whether the histomonads could be maintained through successive transfers on media to which single species of bacteria were added, but they did succeed in infecting turkeys by the rectal inoculation of histomonads grown in what was apparently the initial culture in these media.

In general, *Histomonas meleagridis* appears to require something contained in or liberated by one or more species of bacteria, except that under some conditions the presence of fresh tissue may provide the deficient constituents (Lesser 1961a). The results obtained by Doll and Franker (1963) in their attempts to infect young gnotobiotic turkeys with Histomonas transmitted by feeding embryonated eggs of Heterakis appear to suggest the same thing.

Immunity and Immunization

Natural Immunity. As has already been mentioned, not all gallinaceous birds are equally susceptible to blackhead. Some breeds of chickens possess considerable resistance to the disease, whereas turkeys of all breeds seem to be quite susceptible. Curtice (1907b) observed, "No breed of turkeys thus far tested is immune to the blackhead disease, for all of them, at all ages, so far as tried, have died of it." This is still true. However, some turkeys of all ages have sufficient natural immunity to resist infection as a result of usual exposures, or even fairly heavy experimental inoculations (Lund, 1955). Curtice also noted that "older turkeys apparently resist the disease better than those which are very young, since about 20 per cent of the former have been found to die in the course of the year, and about 90 per cent of the latter." This was a common experience during the early decades of this century. Obviously, two explanations are possible: (1) the most susceptible birds had died before reaching maturity; and (2)

older birds had acquired immunity from previous sublethal or subclinical attacks. Both circumstances may have operated.

By 1951, when management practices were greatly improved, but before the selective use of effective drug control became an extremely common practice, a large-scale study (Bergesen, 1952) revealed that blackhead mortality of young turkeys and that of breeders was about equal, on a per capita basis, about 1.8 per cent. One can, of course, never be sure what proportion of the birds saved as breeders had ever acquired blackhead as poults.

Active Acquired Immunity. Early attempts to demonstrate the development of active acquired immunity under experimental conditions gave variable results. Tyzzer et al. (1921) reported that a turkey that had recovered from experimentally induced blackhead resisted infection by subcutaneous inoculation. However, Tyzzer and Fabyan later reported (1922a, b) that "the protection afforded by a single attack is not permanent." Still later, Tyzzer (1932) reported that histomonads of a pathogenic strain lost their pathogenicity after two years of cultivation in vitro, and that chickens inoculated with organisms from such cultures developed no blackhead when inoculated with pathogenic histomonads. Subsequently (1934, 1936) he reported somewhat comparable results after having used his attenuated strains with turkeys, but observed that the immunity lasted longer when reinforced by exposure of the birds to virulent strains while the immunity produced in response to the original inoculum was still high. Furthermore, he reported that after long continued in vitro cultivation, the attenuated histomonads lost their ability to produce a transient immunity. In general, Tyzzer concluded that from the practical standpoint the development of a vaccine for protecting turkeys from the ravages of blackhead was beset with difficulties, and still far in the future.

With the discovery of drugs effective in the control of blackhead, a new stimulus was given to studies on immunity because turkeys could then be given initial inocu-

lations with virulent histomonads, and still be protected against fatal or extremely severe blackhead, thus assuring the survival of the birds for later challenging. However, the results obtained by testing for immunity in drug-treated birds have varied considerably. Sautter et al. (1950) comment that little immunity was obtained from experimental infections if birds recovered as a result of drug therapy. If any immunity was developed at all, the "turkey must be markedly ill, almost to the point of death, and then recover." DeVolt et al. (1954) obtained essentially the same results. Swales (1950), Kendall (1957), and Clarkson (1963) had some turkeys that resisted Histomonas infections after having recovered from blackhead kept under control by the use of drugs. Apparently immunity is absent or negligible if the drug is used in such a way that little tissue invasion occurs as a result of the initial ("immunizing") infection or infections, but is significant if the drug is not employed until the histomonads are plentiful in the tissues.

Clarkson has demonstrated the presence of serum precipitins as early as seven days after the antigen could be detected in the cecal content, but by this time there was extensive proliferation of histomonads in the cecal mucosa, and the cecal content contained large numbers of organisms. He makes the following pertinent statement: "It is not suggested that the precipitating antibodies demonstrated in turkeys and fowls are a measure of the resistance of the bird to histomoniasis, but, like the protective immune response, serum precipitins are connected with the infection of the cecal mucosa and persist in both turkeys and fowls for a considerable length of time."

Passive Immunization. Attempts to obtain passive immunization by the injection of whole blood from immunized birds (Sautter et al., 1950) or serum from birds showing precipitating antibodies (Clarkson, 1963) have uniformly failed.

Additional Observations and Conclusions. Lund (1959) attempted to immunize

young turkeys by introducing, rectally or with Heterakis eggs, a naturally occurring nonpathogenic histomonad that differs from *Histomonas meleagridis* in several respects. Among these, it does not cause blackhead, and is not known to multiply in the tissues but apparently only in the lumen of the cecum. Rectal inoculations with this histomonad, given three to four weeks before the poults were challenged, afforded some protection against virulent strains of *H. meleagridis* introduced rectally, but virtually none against such organisms introduced with cecal worm eggs. Apparently the immune response was localized in the cecal mucosa immediately in contact with the lumen-dwelling histomonads, and Heterakis larvae readily penetrated this barrier and released their histomonads beyond it. Perhaps Tyzzer's strains of *H. meleagridis*, attenuated by extremely long culture on artificial media, failed to produce an effective immunity for similar reasons.

The search for a means of immunizing chickens and turkeys against blackhead should continue. There is presently no method of immunization of practical use to commercial growers. Consequently, anyone instituting practices designed to protect his birds from blackhead by immunization should be aware that he is definitely experimenting.

Treatment

The treatment of blackhead in turkeys is discussed in Chapter 41, Diseases of Turkeys. In general, the same drugs or classes of substances used with turkeys have been found to have comparable effects with chickens, but less emphasis has been placed on the treatment of chickens because blackhead is less frequently of great economic importance with these birds. As is the case with turkeys, no one method of treatment, nor, indeed, any combination, is completely satisfactory. Control by good management

practices is essential to the success of any program of prophylaxis or therapy.

Many drugs, some closely related to others, and all comprising about half a dozen categories of compounds from the standpoint of chemical constitution, have been used with some success against either the histomonad, the cecal worm, or, in rare instances, both. Wehr et al. (1958) gave an excellent, concise review of the more important of these drugs, their use, and effects. They also included a very useful list of references. Persons interested in pursuing the subject of chemical control in detail should consult this paper and the literature cited therein. Anyone interested merely in treating birds to prevent economic loss should seek information from local sources as to the treatments that have been most successful in his area. Soil structure, climate, local practices, and other considerations may influence the choice of a drug.

Since the appearance of the aforementioned review, there have been reports of the use of certain other substances. Lucas (1961, 1962) and Condren et al. (1962) have reported on tests employing 1,2-dimethyl-5-nitroimidazole, and Lindquist (1962) reported that an antibiotic, Paromomycin sulfate, provided 70 to 80 per cent protection against histomonads introduced by means of Heterakis ova. The effect of the latter substance may be primarily on the intestinal flora, and only secondarily on the histomonads. (See the preceding section on "Cultivation.")

Reid et al. (1960) reported that in controlled experiments a nitrofuran presently used for other purposes suppressed lesions of blackhead in young chickens each of which received about 800 embryonated Heterakis eggs. These workers conclude that the use of this drug in large-scale broiler operations might result in better weight gains and feed conversion if blackhead is present in the flock.

REFERENCES

Ackert, J. E.: 1917. A means of transmitting the fowl nematode, *Heterakis papillosa*. Bioch. Science 44:394.

Bergersen, R. A.: 1952. *Minnesota turkeys—Death losses (Mimeo).* Minn. Turkey Growers Assn

Bishop, A.: 1938. *Histomonas meleagridis* in domestic fowls (*Gallus gallus*). Cultivation and experimental infection. Parasitology 30:181.

Chaddock, T. T.: 1948. Some facts relative to disease as found in wildlife. North Am. Vet. 29.560.

Chester, F. D., and Robin, A.: 1901. Entero-hepatitis or blackhead of fowls. Twelfth Ann. Rep. of Del. Agr. Exper. Sta., p. 60.

Clarkson, M. F.: 1963. Immunological responses to *Histomonas meleagridis* in the turkey and fowl. Immunology 6.156.

Condren, H. B., Davies, R. E., Deyoe, C. W., Creger, C. R., and Couch, J. R.: 1962. Effects of 1,2-dimethyl-5-nitroimidazole on growth and reproduction in turkeys and its residual concentration in tissue. Poultry Sci. 41:1637.

Contiu, I., Rusu, M., and Cioara, M.: 1964. An enzootic of histomoniasis in geese eradicated with tretheon. Probl. zooteh. si Vet. 11:61.

Curtice, C.: 1907a. The rearing and management of turkeys with special reference to the blackhead disease. R.I. Agr. Exper. Sta., Bul 123:1.

———: 1907b. Further experiments in connection with the blackhead disease in turkeys. R.I. Agr. Exper. Sta., Bul. 124.67.

———: 1907c. Notes on experiments with blackhead of turkeys. U.S.D.A. Cir. 119.

Cushman, S. 1893. The production of turkeys. R.I. Agr. Exper. Sta., Bul. 25.

Delappe, I. P.: 1953a. Studies on histomonas. I. Use of antibiotics to facilitate in vitro isolation Exper. Parasit 2 79.

———: 1953b. Studies on histomonas. II. Influence of age of original inoculum and pH on growth in various media. Exper. Parasit. 2:117.

———: 1953c. Studies on histomonas. III. The influence of anaerobic versus aerobic environments on the growth of the organisms in vitro. Exper Parasit. 2:209.

———: 1953d. Studies on histomonas. IV. A continuous automatic potentiometric method of measuring E_h of protozoan cultures. Exper. Parasit 2:280.

Desowitz, R. S.: 1951. Age as a factor influencing fatal infections of histomoniasis in chickens. Jour. Comp. Path. and Therap. 61:231.

DeVolt, H. M.: 1943. A new medium for the cultivation of *Histomonas meleagridis*. Jour. Parasit 29:353.

———, and Davis, C. R. 1936. Blackhead (infectious enterohepatitis) in turkeys, with notes on other intestinal protozoa. Md. Agr. Exper. Sta., Bul. 392.

———, Tromba, F. G., and Holst, A. F.: 1954. An investigation to determine whether immunity to infectious enterohepatitis (blackhead) of turkeys develops during enheptin treatment. Poultry Sci. 33:1256.

Dickinson, E. M.: 1930 Infectious entero-hepatitis in the pea fowl. Jour, Am. Vet. Med. Assn. 76:367.

Doll, J. P., and Franker, C. K: 1963. Experimental histomoniasis in gnotobiotic turkeys, I. Infection and histopathology of the bacteria free host Jour. Parasit. 49:411.

Drbohlav, J.: 1924. The cultivation of the protozoon of blackhead. Jour. Med. Res. 44,677.

Eriksen, S.: 1925 Blackhead in chicks Poultry Sci. 4:250.

Farr, M M.: 1956. Survival of the protozoan parasite *Histomonas meleagridis* in feces of infected birds. Cornell Vet 46 178

———: 1961. Further observations on survival of the protozoan parasite, *Histomonas meleagridis*, and eggs of poultry nematodes in feces of infected birds. Cornell Vet. 51:3.

Franke, J. F.: 1953. A note on the experimental transmission of enterohepatitis of turkeys by arthropods. Canad Jour. Comp. Med 17:250.

Gibbs, B. J.: 1962. The occurrence of the protozoon parasite *Histomonas meleagridis* in the adults and eggs of the cecal worm *Heterakis gallinae*. Jour. Protozool. 9:288.

Glaser, R. W: 1921. On the cytology and life history of the amoebae. Jour. Parasit. 8:1.

Goedbloed, E., and Bool, P. H.: 1962. The protozoan etiology of blackhead. Avian Dis. 6:302.

Graybill, H W: 1921. Data on the development of *Heterakis papillosa* in the fowl. Jour. Exper. Med. 34:259.

———: 1925. Blackhead and other causes of loss of turkeys in California. Univ. Calif. Coll. Agr. Exper. Sta., Circ. 291.

———, and Smith, T.: 1920 Production of fatal blackhead in turkeys by feeding embryonated eggs of *Heterakis papillosa*. Jour. Exper. Med. 31:647.

Green, R G., et al.: 1938. Studies on diseases of quail Minn. Wildlife Dis. Invest. (Mimeo.) 8:160.

Gross, A.: 1930. Progress report of the Wisconsin prairie chicken investigation. Madison, Wis. Animals Branch. Bul. 17.

Higgins, C. H.: 1915. Entero hepatitis or blackhead in turkeys. Canad. Dept. Agr., Health Horron-Smith, C.: 1957. Histomoniasis (blackhead) in poultry. Agr. Rev., Vol. 2 (May), p. 30.

———, and Long, P. L.: 1955 The infection of chickens (*Gallus gallus*) with suspensions of blackhead organism *Histomonas meleagridis*. Vet. Rec. 67:478

———, and Long, P. L.: 1956. Studies on histomoniasis. I. The infection of chickens (*Gallus gallus*) with histomonad suspensions Parasitology 46.79.

Kaupp, B. F.: 1922. Poultry Diseases. 3rd Ed. Alexander Eger, Chicago.

Kendall, S. B.: 1957. Some factors influencing resistance to histomoniasis in turkeys. Brit. Vet. Jour. 133:455.

————: 1959. The occurrence of *Histomonas meleagridis* in *Heterakis gallinae*. Parasitology 49:169.

Kuprowski, M.: 1955. Badania histopatologiczne nad zakaznym zapaleniem jelit ślepych i wątroby indyków-typhlohepatitis infectiosa). Roczn. Nauk. tol. Ser. E 67:69.

Laidlaw, P. P., Dobell, C., and Bishop, A.: 1928. Further experiments on the action of emetine in cultures of *Entamoeba histolytica*. Parasitology 20:207.

Lesser, E.: 1960a. Replacement of serum in the cultivation of *Histomonas meleagridis*. Jour. Parasit. 46:271.

————: 1960b. Cultivation of *Histomonas meleagridis* in a modified tissue culture medium. Jour. Parasit. 46:686.

————: 1961a. In vitro cultivation of *Histomonas meleagridis* free of demonstrable bacteria. Jour. Protozool. 8:228.

————: 1961b. Cholesterol in the cultivation of *Histomonas meleagridis*. Jour. Protozool. 8(Suppl.) 6.

————: 1963. Effect of amphotericin-B on *in vitro* growth of *Histomonas meleagridis*. Jour. Parasit. 49:529.

Lindquist, W. D.: 1962. Some effects of paromomycin sulfate on blackhead in turkeys. Am. Jour. Vet. Res. 23:1053.

Lucas, J. M. S.: 1961. 1,2-dimethyl-5-nitroimidazole, 8595 R. P.: Part I—Prophylactic activity against experimental histomoniasis in turkeys. Vet. Rec. 73:465.

————: 1962. Dimetridazole: Part II. Therapeutic activity and toxicity in the treatment of experimental histomoniasis in turkeys. Vet. Rec. 74:759.

Lund, E. E.: 1955. The progress of histomoniasis (blackhead) in turkeys as related to the size of the infective dose. Poultry Sci. 34:127.

————: 1956. Oral transmission of *Histomonas* in turkeys. Poultry Sci. 35:900.

————: 1958. Growth and development of *Heterakis gallinae* in turkeys and chickens infected with *Histomonas meleagridis*. Jour. Parasit. 44:297.

————: 1959. Immunizing action of a nonpathogenic strain of *Histomonas* against blackhead in turkeys. Jour. Protozool. 6:182.

————: 1960. Factors influencing the survival of *Heterakis* and *Histomonas* on soil. Jour. Parasit. 46(Suppl.):38.

————: 1961. Acquisition and liberation of nonpathogenic histomonads by *Heterakis gallinarum*. Jour. Protozool. 8(Suppl.):6.

————: 1963. *Histomonas wenrichi* n. sp. (Mastigophora:Mastigamoebidae), a nonpathogenic parasite of gallinaceous birds. Jour. Protozool. 10:401.

————, and Burtner, R. H., Jr.: 1957. Infectivity of *Heterakis gallinae* eggs with *Histomonas meleagridis*. Exper. Parasit. 6:189.

————, Wehr, E. E., and Ellis, D. J.: 1963. Role of earthworms in transmission of *Heterakis* and *Histomonas* to turkeys and chickens. Jour. Parasit. 49(Suppl.):50.

Madsen, H.: 1962. On the interaction between *Heterakis gallinarum*, *Ascaridia galli*, "blackhead" and the chicken. Jour. Helminth. 36:107.

Milks, H. J.: 1908. A preliminary report on some diseases of chickens. La. Agr. Exper. Sta. Bul 108:1.

Moore, V. A.: 1896. The direct transmission of infectious enterohepatitis in turkeys. U.S.D.A. Cir. 5.

Niimi, D.: 1937. Studies on blackhead. II. Mode of infection. Jour. Japanese Soc. Vet. Sci. 16:183.

Ohara, T., and Reid, W. M.: 1961. Histomoniasis in chickens: Age of greatest susceptibility and pathogenicity studies. Avian Dis, 5:355.

Reid, W. M., Ohara, T., and Kadunksa, S.: 1960. Controlled experiments with nihydrazone as a blackhead preventive in broilers. Third National Symposium on Nitrofurans in Agr. P. 89.

Riley, W. A., and James, L. G.: 1921. Studies on the chicken nematode, *Heterakis papillosa* Bloch. Jour. Am. Vet. Med. Assn. 59:208.

Sautter, J. H., Pomeroy, B. S., and Roepke, M. H.: 1950. Histomoniasis (entero-hepatitis) in turkeys. II. Chemotherapy of experimental histomoniasis. Am. Jour. Vet. Res. 11:120.

Smith, T.: 1895. An infectious disease among turkeys caused by protozoa (infectious enterohepatitis). Bur. Anim. Ind., U.S.D.A., Bul. 8:1.

————, and Graybill, H. W.: 1920. Blackhead in chickens and its experimental production by feeding embryonated eggs of *Heterakis papillosa*. Jour. Exper. Med. 32:143.

Swales, W. E.: 1948. Enterohepatitis (blackhead) in turkeys. II. Observations on transmission by the cecal worm (*Heterakis gallinae*). Canad. Jour. Comp. Med. 12.97.

————: 1950. Enterohepatitis (blackhead) in turkeys. VII. Experiments on transmission of the disease. Canad. Jour. Comp. Med. 14.298.

Tyzzer, E. E.: 1919. Developmental phases of the protozoon of "blackhead" in turkeys. Jour. Med. Res. 40:1.

Tyzzer, E. E.: 1920a. Observations on the transmission of "blackhead" in turkeys. Jour. Med. Res. 41:219.
———: 1920b. The flagellate character and reclassification of the parasite producing "blackhead" in turkeys—*Histomonas* (gen. nov.) *meleagridis* (Smith). Jour. Parasit. 6:124.
———: 1924. The chicken as a carrier of *Histomonas meleagridis* (blackhead): The protozoon in its flagellated stage. Jour. Med. Res. 44:676.
———: 1926. *Heterakis vesicularis* Frölich, 1791; A vector of an infectious disease. Proc. Soc. Exper. Biol. and Med. 23.708.
———: 1927. Entero-hepatitis in turkeys and its transmission through the agency of *Heterakis vesicularis*. Proc. Third World's Poultry Cong. P. 286.
———: 1932. Problems and observations concerning the transmission of blackhead infection in turkeys. Proc. Am. Phil. Soc. 71:407.
———: 1934. Studies on Histomoniasis, or "blackhead" infection in the chicken and the turkey. Proc. Am. Acad. Arts and Sciences 69:189.
———: 1936. A study of immunity produced by infection with attenuated culture-strains of *Histomonas meleagridis*. Jour. Comp. Path. and Therap. 49.285.
———, and Collier, J. 1925. Induced and natural transmission of blackhead in the absence of Heterakis. Jour. Infect. Dis. 37:265.
———, and Fabyan, M. 1920. Further studies on "blackhead" in turkeys, with special reference to transmission by inoculation. Jour. Infect. Dis. 27:207.
———, and Fabyan, M. 1922a. Practical Suggestions for Raising Turkeys. 2nd Ed., Revised. Mass. Dept. Agr. Bul 15.1.
———, and Fabyan, M.: 1922b. A further inquiry into the source of the virus in blackhead of turkeys, together with observations on the administration of ipecac and sulphur. Jour. Exper. Med. 35:791.
———, Fabyan, M., and Foot, N. C.: 1921. Further observations on "blackhead" in turkeys. Jour. Infect. Dis. 29:268.
Vorobev, M. M., and Kolotilov, I. G.: 1954. Enterohepatitis (histomoniasis) of geese. Ptitsevodstvo 9:35.
Wehr, E. E., Farr, M. M., and McLoughlin, D. K.: 1958. Chemotherapy of blackhead in poultry. Jour. Am. Vet. Med. Assn. 132:439.
Wenrich, D. H.: 1943. Observations on the morphology of *Histomonas* (Protozoa: Mastigophora) from pheasants and chickens. Jour. Morph. 72:279.

LEUCOCYTOZOON INFECTIONS

The genus Leucocytozoon Danilewsky, 1890, is difficult to define, but it is fundamentally very close to Plasmodium and Haemoproteus. Levine (1961), after examining the life cycles as reported for representatives of each of the three genera, concluded that "there is no point in retaining more than a single family in the suborder" (which he designated as Hemospororina). But Fallis and Bennett (1961), after comparing several stages in the life cycles of members of the three genera, proposed that each genus occupy a separate family, all being in the order Hemosporidiida. Variations in interpretations appear to rest largely on the relative significance ascribed to similarities and differences in the life cycles. The similarities are, of course, impressive.

The gametocytes of Leucocytozoon, supposedly without pigment, appear in the circulating blood inside distorted host cells, presumably leucocytes, and multiplication is by schizogony undergone only in the internal organs. As in the malarias, there is a blood-sucking dipterous intermediate host in which fertilization and sporogony take place, very much as in the case of the true malarial parasites. There has been considerable difference of opinion over the nature of the host cell of the gametocytes, but Huff (1942) found evidence that in *Leucocytozoon simondi*, while various types of cells are initially invaded, this stage becomes fully grown only in "monocytes, or more possibly macrophages." Hartman (1929) and O'Roke (1934) and others have observed pigment granules in the cytoplasm. Huff (1942), however, has pointed out that such granules may not be true hematin such as occurs in malarial parasites that develop in erythrocytes, because it is not visible as pigment in unstained preparations and its optical properties are different from those of malarial pigment. Borg (1953) could remove the pigment from *L. mansoni* with dilute acetic acid and ammonia, but not from Haemoproteus. The nucleus of the male gameto-

cyte is somewhat larger than that of the female and stains less deeply, and its cytoplasm also stains less deeply. (See illustration of Leucocytozoon in Chapter 41.)

Birds seem to be the sole hosts of Leucocytozoon. Coatney (1937) has cataloged and host-indexed 68 species up to that year. The card files of the U.S.D.A. Index Catalog of Medical and Veterinary Zoology list almost 100 species of Leucocytozoon, but perhaps a quarter of these are now regarded as synonyms, or are otherwise out of use. At least two, and probably three, species occur in domestic birds in North America. Leucocytozoon simondi occurs in domestic ducks and geese, and has been reported from many wild anseriform birds. Levine and Hanson (1953) tabulated reports of this parasite from 23 species of wild waterfowl. Leucocytozoon smithi occurs in domestic and wild turkeys. Atchley (1951) described Leucocytozoon andrewsi, n. sp., from chickens in South Carolina. Levine (1961) regards this as a synonym of L. caulleryi, a species reported by several investigators as present in chickens in southern and eastern Asia. Wehr (1962) observed, "The status of L. andrewsi is in doubt, and cannot be determined until more is known of its development and mode of transmission in the chicken."

L. simondi Mathis and Leger, 1910, first described from an oriental teal in Tonkin, is found in the blood as gametocytes measuring 14μ–15μ by 4.5μ–5.5μ inside for the most part, elongate spindle-shaped host cells pointed at both ends and about 48μ in length. The parasite either lies beside a conspicuously staining bar or between two such bars representing the nucleus of the host cell. Huff identifies the latter as a distorted monocyte or macrophage. Fallis et al. (1951) observed round gametocytes in addition to the spindle-shaped, which were mature because of the ability to exflagellate. L. anatis Wickware (1915), described from tame ducks, appears on morphological grounds to be a synonym of L. simondi. (See Coatney and Roudabush, 1937; Herman, 1938.) L. anseris Knuth and Magdeburg, 1922, described

from geese, appears to be another synonym. Fallis et al. (1954) found in cross-infection trials that ducks and geese were suitable hosts for this species, but chickens, turkeys, pheasants, and ruffed grouse were not.

Schizogony takes place in such internal organs as the liver, heart, brain, spleen, and lungs. According to Huff (1942), the earliest stages consist of small ovoid bodies inside macrophages, extracellular, or within liver (parenchyma) cells, and showing some degree of separation of their more densely staining material. The "hepatic schizonts" in liver cells, measuring 11μ–18μ, undergo differentiation into, first, cytomeres, and these in the final step in schizogony break up into small merozoites. The "megaloschizonts," measuring 60μ–105μ when mature, mostly develop inside cells, possibly lymphoid cells or macrophages, within or outside blood vessels. The earliest stages observed were inside cells and were already multinuclear. Later stages were very large and contained numerous cytomeres (intermediate subdivisions of a schizont) and a large, conspicuous "central body" concerning whose true nature Huff is not certain, but he conjectures that it is the hypertrophied nucleus of the host cell. (Cowan, 1955, on the other hand, interprets the "central body" as an integral part of the parasite—a "primordium"—from whose surface bud off the primary cytomeres.) In the last step of schizogony the megaloschizont contains many thousands of bipolar merozoites being released into the gametocytes after invading suitable cells. Fallis et al. (1951) noted mature forms in peripheral blood as early as 7 days after exposure to natural infection, and later Fallis et al. (1956), 5 to 6 days after. Parasitemia may terminate in about 30 days of its first appearance (Chernin, 1952c).

O'Roke (1931) first showed a blood-sucking fly, Simulium venustum, to be the vector among ducks in Michigan and outlined the development in this insect. Fallis et al. (1951, 1956) have added S. croxtoni, S. euradminiculum, and S. rugglesi to the list of transmitters, with the suggestion

that the latter is the natural vector in Canada. Development in the fly may be completed in a minimal time of 4 days and a fly may remain infective for as long as 18 days.

There is no doubt about the pathogenicity of *L. simondi* in ducks and geese. O'Roke in Michigan noted 35 per cent mortality in an outbreak among ducks, confirming in general the observations of Wickware (1915) in Canada, while Knuth (1922) and Knuth and Magdeburg (1922) reported on a serious and often fatal disease among young geese in Germany apparently caused by the same organism. (See also Stephan, 1922.) About 68 per cent of the fatalities occur from 11 to 19 days after exposure (Chernin, 1952a). Some of the pathological effects of the disease are anemia, leucocytosis, splenomegaly, and liver degeneration and hypertrophy (Fallis *et al.*, 1951). Extensive tissue damage was noted by Huff (1942) in the spleens and hearts of ducks carrying megaloschizonts. O'Roke (1930), Chernin and Sadun (1949), and Chernin (1952a) have noted that the greatest number of infections in Northern Michigan is coincident with the hottest part of the summer and occurs mostly in July.

O'Roke (1934) and Huff (1942) both observed that the gametocytes decrease in number in the blood until in midwinter they had either disappeared or become scarce, and that they reappeared in the spring. The sporadic occurrence of parasites in the blood during the entire winter, however sparse they may be, indicates that sporogony is proceeding at a low level. After a three-year investigation of the spring relapse phenomenon, Chernin (1952b) concluded that it was associated with the onset of renewed reproductive activity in both sexes of the avian host. Female ducks subjected to increased hours of artificial light per day in the fall and winter not only commenced egg laying earlier, but experienced earlier onset of the relapse parasitemia.

L. smithi Laveran and Lucet, 1905, was first seen in turkeys in eastern United States by Theobald Smith (1895), after whom it is named. It has been reported by Volkmar (1929) as occurring in North Dakota and Minnesota, by Skidmore (1932) in Nebraska, by Johnson (1942, 1945) in Virginia, and Hinshaw and McNeil (1943) in California. Other reports indicate its presence in France, Germany, Crimea, and Canada. The parasite in general resembles *L. simondi* of Anseriformes, but turkeys are probably not susceptible to the latter (Fallis *et al.*, 1954; Byrd, 1959). Terrific losses have been experienced among poults, and even adults, e. g., Stoddard *et al.* (1952) reported an outbreak in Georgia wherein a grower suffered a 75 per cent loss in a flock of 1600 five-month-old turkeys presumably due to Leucocytozoon disease. Flies of the genus *Simulium* were observed in the vicinity of roosts and feeding troughs. The widespread occurrence of the parasite in certain areas is emphasized by the survey of Travis *et al.* (1939) on adult domesticated turkeys during which 289 of 357 were found infected in Georgia, 60 of 67 in Florida, 4 of 12 in Alabama, and 7 of 9 in South Carolina. Mosby and Handley (1943) reported the parasite in 40 per cent of 268 turkeys in Virginia, of which 40 were domestic, 183 were captivity-reared wild turkeys, and 45 were wild. Others who have reported Leucocytozoon in captive wild turkeys are Johnson *et al.* (1938), Wehr and Coburn (1943), and Travis *et al.* (1939), while Kozicky (1948) found it in all of 5 native wild turkeys taken in the field in Pennsylvania.

Byrd (1959), who studied Leucocytozoon in pen-raised and free-ranging wild turkeys in the Cumberland State Forest of Virginia, reported incidences of almost 100 per cent among mature birds maintained in large, open pens in the spring. However, he observed, "Heavy infections, comparable to those reported for *L. simondi* in ducks, do not seem to occur," and "Few symptoms were observed in wild turkeys that could be attributed to the disease." He attempts to relate this lack of pathogenicity to local factors, such as the time at which suitable vectors were prevalent, and the age of the

birds at first exposure. With regard to the vector, Byrd observed, "The period of infection for the disease on the Cumberland forest is such that *P. [Prosimulium] hirtipes* appears to be the only species responsible for its transmission." Byrd considers the possibility, also, that wild turkeys may "have developed some degree of natural resistance to the disease" (leucocytozoonosis).

Skidmore considered *Simulium occidentale* to be the vector concerned in a Nebraska outbreak of leucocytozoonosis in domestic turkeys, while Johnson (1938) found *S. nigroparvum* to be the vector in Virginia. Wehr (1962) studying outbreaks of Leucocytozoon infection in domestic turkeys in South Carolina, transmitted the parasite experimentally by intramuscular injections of suspensions of ground blackflies, *Simulium slossonae*. He cites communications with others working in the same region who also found this species of blackfly to transmit the disease. Wehr, studying schizogony in the domestic turkey, found indications that the life cycle of *L. smithi* may differ in some details from that of *L. simondi* in ducks. These observations were in agreement with those of some earlier workers, such as Richey and Ware (1955) and Newberne (1955).

The single instance in which Leucocytozoon has been reported from chickens in North America (Atchley, 1951) gives no indication of the pathogenicity of the parasite in this host. Levine (1961), who regards Atchley's organism as having probably been *L. caulleri*, concludes, "This species is presumably pathogenic, but accounts of it have been so mixed up with those of *L. sabrazesi* (a species thus far known only from southeast Asia) "that its pathogenicity is uncertain."

L. sabrazesi Mathis and Leger, 1910, was frequently found by its describers in chickens in Tonkin. Kuppusamy (1936) considers it a pathogen of *economic* importance in Malay. The gametocytes in the blood, like *L. simondi*, are enveloped in elongate spindle-shaped host cells, pointed at both ends. *L. schüffneri* Prowazek, 1912,

from domestic fowl in Sumatra, may be a synonym. *L. caulleryi* Mathis and Leger, 1909, was also observed in the blood of chickens in Tonkin. The gametocytes were enveloped in round host cells which, when the parasites were mature, were frequently without nuclei.

L. andrewsi is a species with rounded gametocytes described by Atchley (1951), who found it in 15.3 per cent of 400 chickens examined in South Carolina. This parasite may be synonymous with *L. caulleryi*, but the describer considered the regular presence of the nucleus in the host cell sufficient basis for the creation of the new species.

L. mansoni Sambon, 1908, from the Swedish capercaillie *(Tetrao urogallus)* has been the subject of an intensive investigation by Borg (1953), whose results led him to conclude that this and similar parasites occurring in Swedish forest game birds have no significant role in the widespread mortality in their hosts (capercaillie, black grouse, hazel grouse). Gametocytes were of three types — round, oval, and elongated. The host cells of the latter two types had tails at the two extremities unless they were worn off.

Treatment. Drug treatment has been reviewed by O'Roke (1934) and Coatney and West (1937). Pamakin (plasmochin) proved unsatisfactory, but quinine showed promise if fed for a time before adult gametocytes showed in the blood, but did not affect adult gametocytes. Coatney found that atabrin did attack the adult gametocytes. On the other hand, Fallis (1948) had no success in curing or suppressing infections of *L. simondi* in ducks with paludrine, atabrin, or sulfamerazine. He was inclined to blame the resistance of the tissue stages for the failure.

Control. Control is only to be attained through management. This means, principally, that duck and turkey culture should not be attempted in regions where there is running water serving as breeding places for an abundance of black flies (Simulium). Otherwise, it is necessary to screen the young ducks from the flies,

which is difficult. Removing parasitized young and adult birds from the flock would also prove helpful in some instances. Since the young are more susceptible to the disease than the adults, it would help also to control the disease if ducklings were hatched either before or after the main black fly season (see O'Roke, 1934).

Anthony and Richey (1958) attempted to control outbreaks of *L. smithi* in domestic turkeys in South Carolina by spraying the breeding grounds of the black flies with DDT. This was very effective for two weeks and moderately so for another two weeks. After that, near drought conditions developed, so black fly control actually averaged almost 95 per cent effective over a 10-week period. Nevertheless, poults placed on range four weeks after the application of DDT to the black fly breeding grounds started showing Leucocytozoon disease within two weeks of release. Within another 4 weeks, 80 to 100 per cent of the birds were infected. Anthony and Richey suggested that other diptera may have been responsible for the transmission of the disease.

REFERENCES

Anthony, D. W., and Richey, D. J.: 1958. Influence of black fly control on the incidence of *Leucocytozoon* disease in South Carolina turkeys. Jour. Econ. Entomol. 51:845.

Atchley, F. O.: 1951. *Leucocytozoon andrewsi* n. sp. from chickens observed in a survey of blood parasites in domestic animals in South Carolina. Jour. Parasit. 37:483.

Borg, K.: 1953. On Leucocytozoon in Swedish capercaillie, black grouse and hazel grouse. Seringska Boktryckeriet, Lund, 109 pp.

Byrd, M. A.: 1959. Observations on *Leucocytozoon* in pen-raised and free-ranging wild turkeys. Jour. Wildlife Mgt. 23:145.

Chernin, E.: 1952a. The epizootiology of *Leucocytozoon simondi* infections in domestic ducks in Northern Michigan. Am. Jour. Hyg. 56:39.

——— 1952b. The relapse phenomenon in the *Leucocytozoon simondi* infection of the domestic duck. Am. Jour. Hyg. 56:101.

——— 1952c. Parasitemia in primary *Leucocytozoon simondi* infections. Jour. Parasit. 38:499.

——— and Sadun, E. H.: 1949. *Leucocytozoon simondi* infections in domestic ducks in northern Michigan with a note on transmission. Poultry Sci. 28:890.

Coatney, G. R.: 1937. A catalog and host-index of the genus Leucocytozoon. Jour. Parasit. 23:202.

——— and Roudabush, R. L.: 1937. Some blood parasites from Nebraska birds. Am. Midl. Nat. 18:1005.

——— and West, E.: 1937. Some notes on the effect of atabrin on gametocytes of the genus Leucocytozoon. Jour. Parasit. 23:227.

Cowan, A. B.: 1955. The development of megaloschizonts of *Leucocytozoon simondi* Mathis and Leger. Jour. Protozoology 2:158.

——— 1957. Reactions against the megaloschizonts of *Leucocytozoon simondi* Mathis and Leger in ducks. Jour Immunol. Dis. 100:82.

Fallis, A. M.: 1948. Observations on *Leucocytozoon* infections in birds receiving paludrine, atabrin, and sulphamerazine. Canad. Jour. Res., D 26:73.

——— Anderson, R. C., and Bennett, G. F.: 1956. Further observations on the transmission and development of *Leucocytozoon simondi*. Canad. Jour. Zool. 34:389.

——— and Bennett, G. F.: 1961. Sporogony of *Leucocytozoon* and *Haemoproteus* in simuliids and ceratopogonids and a revised classification of the Haemosporidiidia. Canad. Jour. Zool. 39:215.

——— Davies, D. M., and Vickers, M. A.: 1951. Life history of *Leucocytozoon simondi* Mathis and Leger in natural and experimental infections and blood changes produced in the avian host. Canad. Jour. Zool. 29:305.

——— Pearson, J. C., and Bennett, G. F.: 1954. On the specificity of Leucocytozoon. Canad. Jour. Zool. 32:120.

Hartman, E.: 1929. The asexual cycle in *Leucocytozoon anatis*. Jour. Parasit. 15:178.

Herman, C. M.: 1938. *Leucocytozoon anatis* Wickware, a synonym for *L. simondi* Mathis and Leger. Jour. Parasit. 24:472.

Hinshaw, W. R., and McNeil, E.: 1943. *Leucocytozoon* sp. from turkeys in California. Poultry Sci. 22:268.

Huff, C. G.: 1942. Schizogony and gametocyte development in *Leucocytozoon simondi*, and comparisons with Plasmodium and Haemoproteus. Jour. Infect. Dis. 71:18.

Johnson, E. P.: 1942. Further observations on a blood protozoan of turkeys transmitted by *Simulium nigroparvum* (Twinn). Am. Jour. Vet. Res. 3:214.

——— 1945. Blood parasites of turkeys. Mich. St. Coll. Veterinarian 5:121.

——— Underhill, G. W., Cox, J. A. and Threlfeld, W. L.: 1938. A blood protozoan of turkeys transmitted by *Simulium nigroparvum* (Twinn). Am. Jour. Hyg. 27:649.

Knuth, P.: 1922. Demonstration über in Deutschland gefundene Leucocytozoen der Hausgans. Arch. Schiffs.- u. Trop.-Hyg. 19:185.

———, and Magdebutg, F.: 1922. Ueber ein durch Leucocytozoon verursachtes Sterben junger Gänse. Berliner tierärztl. Wochenschr. 38:359.

Kozicky, E. L.: 1948. Some protozoan parasites of the eastern wild turkey in Pennsylvania. Jour. Wildlife Mgt. 12:263.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn. 412 pp.

———, and Hanson, H. C.: 1953. Blood parasites of the Canada goose, Branta canadensis interior. Jour. Wildlife Mgt. 17:185.

Mathis, C., and Leger, M.: 1910. Leucocytozoon d'une tourterelle (Turtur humilis) et d'une sarcelle (Querquedula crecca) du Tonkin. Compt. Rend. Soc. Biol., Paris 68:118.

Mosby, H. S., and Handley, C. O.: 1943. The wild turkey in Virginia: its status, life history, and management. Publ. by Commission of Game and Inland Fisheries, Richmond, Va., 281 pp.

Newberne, J. W.: 1955. The pathology of Leucocytozoon infection in turkeys with a note on its tissue stages. Am. Jour. Vet. Res. 16:593.

O'Roke, E. C.: 1930. The incidence, pathogenicity, and transmission of Leucocytozoon anatis of ducks. Jour. Parasit. 17 (Suppl. 2):112.

——— 1934. A malaria-like disease of ducks caused by Leucocytozoon anatis Wickware. Univ. Mich. School Forestry and Conserv., Bul. No. 4.

Richey, D. J., and Ware, R. E.: 1955. Schizonts of Leucocytozoon smithi in artificially infected turkeys. Cornell Vet. 45:642.

Skidmore, L. V.: 1932. Leucocytozoon smithi infection in turkeys and its transmission by Simulium occidentale Townsend. Zentralbl. f. Bakt. I. Orig. 125:329.

Smith, T.: 1895. An infectious disease among turkeys caused by Protozoa (infectious enterohepatitis). Bur. Anim. Ind., U.S.D.A., Bul. 8:1.

Stephan, J.: 1922. Über eine durch Leucocytozoon verursachte Gänse und Putenerkrankung. Deutsch. tierärztl. Wochenschr. 30:589.

Stoddard, E. D., Tumlin, J. T., and Cooperrider, D. E.: 1952. Recent outbreak of Leucocytozoon infection in adult turkeys in Georgia. Jour. Am. Vet. Med. Assn. 121:190.

Travis, B. V., Goodwin, M. H., Jr., and Gambrell, E.: 1939. Preliminary note on the occurrence of Leucocytozoon smithi Laveran and Lucet (1905) in turkeys in the southeastern United States. Jour. Parasit. 25:278.

Volkmar, F.: 1929. Observations on Leucocytozoon smithi; with notes on Leucocytozoa in other poultry. Jour. Parasit. 16.24.

Wehr, E. E.: 1962. Studies on leucocytozoonosis of turkeys, with notes on transmission, and control of Leucocytozoon smithi. Avian Dis. 6:195.

———, and Coburn, D. R.: 1943. Some economically important parasites of the wild turkey and Hungarian partridge of Pennsylvania. Game News 13:14.

Wickware, A. B.: 1915. Is Leucocytozoon anatis the cause of a new disease in ducks? Parasitology 8:17.

TOXOPLASMA INFECTIONS

Toxoplasmosis is an infection of mammals and birds with *Toxoplasma gondii* Nicolle and Manceaux, 1909, whose type host is the rodent *Ctenodactylus gundi*, the gundi of North Africa. This microorganism is known to occur naturally in at least 24 genera of mammals belonging to 7 orders and a number of species of birds of several orders (Manwell and Drobeck, 1953a), and, perhaps, in certain reptiles also (Fig. 37.6). Some of these hosts (Jacobs, 1956) are moles, gundis, guinea pigs, mice (but laboratory strains do not ordinarily harbor the organism), rats, rabbits and hares, dogs, foxes, cats, swine, sheep, cattle, baboons, chimpanzees, monkeys, man, pigeons, and chickens. Capercaillie,

black grouse, hazel grouse (Borg, 1953), crows (Finlay and Manwell, 1956), and barnyard geese (Biering-Sorensen [1957] dye test) may be added to this list. Interest in the parasite increased sharply about 1939 when its importance in man became evident. Thereafter interest in toxoplasmosis in all animals burgeoned, partly because of its potentially serious and multifarious effects on human beings and the epidemiological community of interest people share with animals in its dissemination, and partly because of its actual or potential role in causing losses among livestock. Expanding interest reflected in publications on the subject required 2,000 titles by 1956 (Hoare), most of which are listed in two bibliographies (Eyles and Frenkel, 1952, 1954). (See also Markham, 1956.)
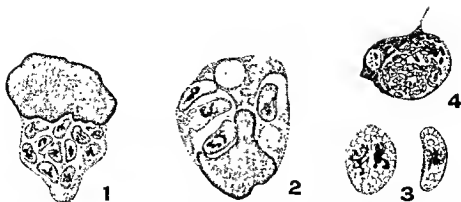
FIG. 37.6 -- 1, 2--Toxoplasma bodies in lymphoid cells of a ground squirrel. 3--Free Toxoplasma in same host, individual on left in division. 4--Toxoplasma in cytoplasm of nerve cell of rabbit. (1-3, after Sossuchin; 4, after Levaditi et coll.)

Present interest is restricted to avian toxoplasmosis, but more general information is available in reviews by Hoare (1956), Jacobs (1953), Manwell and Drobeck (1953a, b), Brooke (1955), and the 1956 symposium papers (in order) by Markham, Jacobs, Feldman and Miller, Siim, Eichenwald, Frenkel, and Eyles.

The taxonomic position of Toxoplasma remains uncertain. Wenyon (1926) placed it among organisms of doubtful nature, perhaps not protozoal at all. Manwell and Drobeck (1953a) and Ludvík (1956) have indicated ways in which it resembles Sarcocystis. Goldman et al. (1957) have pointed out structural similarities in Toxoplasma gondii and Besnoitia jellisoni, although they found these two organisms to be serologically distinct. Levine (1961) considered Toxoplasma as belonging to the same order as Sarcocystis, but separated the two at the family level, placing Toxoplasma in the same family as Besnoitia and Encephalitozoon. Westphal (1954) regarded Toxoplasma as a nonflagellated, kinetoplast-free leishmanial type, and suggested that it is a member of the Trypanosomidae, but this view has apparently received little support.

The trophozoite of Toxoplasma is a crescentic body, less rounded at one end than the other, measuring 4μ-7μ in length and 2μ-4μ in width. The nucleus is nearest the thicker end. An electron microscope

study (Gustafson et al., 1954) has revealed a small hollow cone at the more tapering end, and, radiating from the cone's base toward the thicker end, 14 to 16 homogenous fibrils. The toxoplasm is capable of several types of movement of translation (Manwell and Drobeck, 1953a). Reproduction is principally by binary fission of this stage inside a vacuole in one of a considerable variety of host cells — macrophages, lymphocytes, parenchymal cells of liver, adrenals, lungs and brain, microglia, neuroglia, etc. A form of internal budding (endodyogeny) was described by Goldman et al. (1958), and this was in part confirmed by Gangi and Manwell (1961). However, the latter investigators did not see all stages characteristic of endodyogeny, and they made the interesting observation, "the inability to demonstrate cytochemically DNA in the parasites stained with silver protein suggests that morphologic structures seen in endodyogeny are not primarily involved with the distribution of gametic material." Repeated binary fissions result in an accumulation of toxoplasms, which has been called a terminal colony, occupying the greater part of the host cell. The members of the colony are released by rupture of the cell and invade other cells. Certain workers, apparently with good evidence, insist that schizogony also occurs but that it is unusual (see Pereira de Castro, 1955). In the chronic or

latent infection a cystlike stage is characteristic and predominant. It is a round or oval agglomeration of toxoplasma, usually found in the brain, measuring $14.5\mu–37.7\mu$ across and enclosed by a wall of sufficient tenacity to hold the structure together when the surrounding tissue is disintegrated by shaking with glass beads (Rodhain, 1950). Jacobs et al. (1960) reviewed the recent literature dealing with these cystlike bodies and concluded that they may properly be called "cysts" rather than "pseudocysts," as some have suggested. They also demonstrated that the cyst wall was destroyed immediately on contact with a peptic digest solution heated to 37° C., and that the toxoplasmas so liberated remained infective for mice after exposure to the pepsin·HCl solution $1\frac{1}{2}$ to 2 hours, but not after exposures of 3 hours or more. The cyst wall was digested in 1 per cent trypsin, also, and toxoplasmas remained viable in this solution after 6 hours. Proliferative toxoplasmas did not withstand the action of artificial gastric juice for even very brief intervals.

The manner in which toxoplasmosis is spread is unknown. Congenital transmission has positively been established in such mammals as human beings (Feldman and Miller, 1956), dogs (Koestner and Cole, 1960), pigs, and cattle. Presumably in such cases the mother was experiencing an acute attack, either the primary one or an exacerbation of a chronic infection. The possibility of filth transmission exists because the parasites have been found in the feces, urine, and other bodily eliminations of infected animals, though corroborative experimental evidence of it is mostly lacking. The organisms have been found in cow's milk. The ingestion of infested flesh is another likely possibility, particularly the flesh of chronic cases where pseudocysts are present. The susceptibility of the parasites to drying, freezing, moderate heat, gastric juice, and even under ordinary conditions to such fluids as physiological salt solution and culture media outside the animal body militates against the contact or filth-borne hypothesis, but does not al-together exclude it. Venereal transmission is another possibility, but again corroboration is lacking.

Transmission by blood-sucking arthropods such as ticks, mites, mosquitoes, fleas, and lice has been suggested and deserves a certain degree of plausibility because of the occurrence of the parasites in the circulating blood, usually inside monocytes. Wolfson (1941) worked with a strain which she obtained from a worker who had first observed it in a guinea pig which had been "injected with some ticks." A limited number of successful experimental transmissions with certain ticks and a species of louse lend further credence to the hypothesis (Woke et al., 1953).

The first report of spontaneous toxoplasmosis in chickens was that by the German veterinarian Hepding (1939), who found the organisms, which he considered secondary invaders, in the retina of a hen with neurolymphomatosis. Other early observers are mentioned by Erichsen and Harboe (1953a). The latter workers studied an outbreak with some fatal illnesses in a small flock of White Leghorns in southern Norway. The outstanding symptoms of the sick birds were anorexia, emaciation, paleness and shrinking of the comb, and, in some instances, diarrhea and blindness. Nine birds from the flock were necropsied and of these Toxoplasma was observed in sections of the organs of five, but not in the eye despite the diffuse chorioiditis. In an important later study by the same authors, two experimentally infected birds and one naturally infected bird were found to have developed multiple gliomas in their brains. Intracerebral or intraperitoneal injections of suspensions of tissue from three birds resulted in Toxoplasma infection in three mice. The most outstanding lesions were focal and diffuse pericarditis, myocarditis, necrotizing encephalitis, necrotic hepatitis, and ulcerative gastroenteritis. The dye tests were either negative or feebly positive.

A rapidly developing outbreak (in Brazil), in which the total mortality was 50 per cent and almost the entire flock was

affected, was reported by Nobrega et al. (1955). Symptoms were similar to those of coccidiosis except that the stools were whitish. Outstanding necropsy findings were enlarged liver and spleen with necrotic foci, heart pale with yellowish-white nodules of various sizes, and lungs with extensive areas of congestion and consolidation. Toxoplasma was observed in 10 of 15 chicks examined, both in smears of lungs, liver, and spleen stained with Giemsa and in tissue sections of spleen, heart, and lungs, but with one exception not in the central nervous system. The parasite was demonstrated in mice four to six months after intracerebral inoculation with organ suspensions.

Biering-Sorensen (1956) in Denmark diagnosed toxoplasmosis in 35 hens from 21 flocks out of 26,000 fowls necropsied. Of these, 11 showed necrosis of the optic chiasma. In an epidemiological study of an area where toxoplasmosis had been found in fowls, the same author (1957) found 15 of 57 hens, all of 6 human beings, 4 of 22 geese, 2 of 6 horses and 7 of 8 pigs positive to the dye test. Furthermore, mouse inoculation tests showed 8 fowls to be harboring the parasite. Pseudocysts were demonstrated in 8 fowls.

A number of workers have reported the natural occurrence of toxoplasmosis in the common pigeon (Carini, 1911; Reis, Nobrega, and Reis, 1936; Nobrega and Reis, 1942; Springer, 1942; Johnson, 1944; Feldman and Sabin, 1949; Wiktor, 1950; Manwell and Drobeck, 1951; Jacobs et al., 1952). The last-named authors found positive evidence of toxoplasma infection in 10 of 80 pigeons trapped in Washington, D.C. In a study of experimental toxoplasmosis in pigeons, Jacobs et al. (1953) demonstrated that antibody persists in the blood of birds infected with strains of low virulence for shorter periods of time than in birds infected with a strain of high virulence.

Diagnosis. (See Brooke, 1955; Eichenwald, 1956; Jacobs et al., 1952, 1953; Westphal, 1949; Westphal and Palm, 1953, 1954.) According to Sabin (1939), the

capacity to multiply and produce disease in a variety of hosts, including mammals and birds, is to be regarded as the chief taxonomic characteristic of Toxoplasma. Morphology as the only guide may be confusing (see also Wolfson, 1940). For these reasons, intracerebral (0.03 ml.) and intraperitoneal (0.1 ml.) injections of body fluids and suspensions of tissues (brain, liver, lungs, spleen, etc.) of suspect hosts into laboratory mice, hamsters, guinea pigs, or young chicks, and subsequent microscopic examinations of both fresh and stained smears of the inoculated hosts, is depended upon both for confirmation of positive microscopic diagnosis and for revealing the presence of the etiological agent in cases where the microscopic examination is negative. Of course, the actual demonstration of the parasite in the original case is in itself a valuable diagnostic accomplishment. Positive animal transmission adds much to the reliability of the diagnosis. When ascites or other symptoms of illness develop in the experimental host, the peritoneal fluid and smears of brain, liver, lungs, spleen, etc., should be examined for the parasite. If no parasites are found, or if the inoculated animals do not show symptoms in 7 or 8 days, blind passages should be made from the inoculated animals to another series. With chickens and pigeons, a large number of blind subpassages in laboratory mice may be required before the parasite can be observed microscopically, or it may not be demonstrated at all.

Direct impression smears of tissues or smears of peritoneal fluid stained in Giemsa, or tissue sections of brain, lung, liver, spleen, lymph nodes, eye, etc., often serve for the direct microscopic observation of Toxoplasma when the oil-immersion lens is employed. Rarely, the parasites are seen in blood smears, inside monocytes, or free.

There are at least four serological tests: the rabbit skin neutralization test, the complement-fixation test, the skin test, and Sabin and Feldman's (1948) alkaline methylene blue dye test, simply called the

dye test. The latter is the most practical under a variety of conditions. Unfortunately, infected chickens often respond but weakly to the dye test, but pigeons perform better, except in infections of long standing.

Symptomatology and necropsy findings alone are not sufficiently reliable to be depended upon for final diagnosis.

Cultivation. Cook and Jacobs (1958) tabulated the history of *in vitro* cultivation of Toxoplasma. Kaufman and Maloney (1962) studied the organism's multiplication in tissue culture, and suggested that certain relationships may exist between the rate of multiplication of a strain of Toxo-

plasma and its susceptibility to antimetabolic drugs such as pyrimethamine and the sulfonamides.

Treatment. (See Eyles, 1956.) Both sulfonamides (especially sulfadiazine, sulfamethazine, sulfamerazine, and sulfapyrazine) and the 2,4-diamino pyrimidines (especially pyrimethamine = Daraprim) have been shown to be effective in treating mouse and human infection. Eyles and Coleman (1953) discovered that Daraprim and sulfonamides act synergistically, so that it was possible to obtain chemotherapeutic effect with much lower combined dosages than with the two drugs separately.

REFERENCES

Biering-Sorensen, U.: 1956. Fjerkraetoxoplasmose. Nordisk. Vet.-Med. 8:140.
———: 1957. Serologiske undersøgelser over udbredelsen af latent toxoplasmose hos dyr og mennesker i et milieu med verificeret toxoplasmosis gallinarium. Nordisk. Vet.-Med. 9:129.
Borg, K.: 1933. On Leucocytozoon in Swedish capercaillie, black grouse and Hazel grouse. 100 pp. Berlingska Boktryckeriet, Lund.
Brooke, M.: 1954. The laboratory diagnosis of toxoplasmosis. Official Bul. Conf. St. and Prov. P. H. Lab. Directors, Communicable Dis Center, P. H. Service, Atlanta, Ga. 12:109.
———: 1955. Toxoplasmosis. Mississippi Doctor Nov:160.
Carini, A.: 1911. Infection spontanée du pigeon et du chien due au *Toxoplasma cuniculi.* Bul. Soc. Path. Exot. 4(8):518.
Cook, M. K., and Jacobs, L.: 1958. Cultivation of *Toxoplasma gondii* in tissue cultures of various derivations. Jour. Parasit. 44:172.
Coutelen, F., Biguet, J., Doby, J. M., and Deblock, S.: 1953. Le problème des toxoplasmes aviaires. Réceptivité variable de quelques oiseaux à une souche humaine de toxoplasmes. Ann. de Parasit. 28:129.
Cross, J. B.: 1947. A cytologic study of toxoplasma with special reference to its effect on the host's cell. Jour. Infect. Dis. 80:278.
Drobeck, H. P., Manwell, R. D., Bernstein, E., and Dillon, R. D.: 1953. Further studies of toxoplasmosis in birds. Am. Jour. Hyg. 58 329.
Eichenwald, H. F.: 1956. The laboratory diagnosis of toxoplasmosis. Ann. N.Y. Acad. Sci. 64:207.
Erichsen, S., and Harboe, A.: 1953a. Toxoplasmosis in chickens. I. An epidemic of toxoplasmosis in a chicken flock in southeastern Norway. Acta Path. et Microbiol. Scandinavica 33:56.
———, and Harboe, A.: 1953b. Toxoplasmosis in chickens. II. So-called gliomas observed in chickens infected with toxoplasma. Acta Path. et Microbiol. Scandinavica 33:381.
Eyles, D. E.: 1956. Newer knowledge of chemotherapy of toxoplasmosis. Ann. N.Y. Acad. Sci. 64:252.
———, and Coleman, N.: 1953. Synergistic effect of sulfadiazine and daraprim against experimental toxoplasmosis in the mouse. Antibiot. and Chemotherap. 3.483.
———, and Frenkel, J. K.: 1952. A bibliography of toxoplasmosis and *Toxoplasma gondii* Pub. Health Serv. Publ. No. 247. Washington, D.C.
———, and Frenkel, J. K.: 1954. A bibliography of toxoplasmosis and *Toxoplasma gondii.* First Suppl. Natl. Microbiol. Inst., Bethesda, and Univ. of Kansas, Lawrence.
Feldman, H. A., and Miller, L. T.: 1956. Congenital human toxoplasmosis. Ann. N.Y. Acad. Sci. 64:180.
———, and Sabin, A. B.: 1949. Skin reactions to toxoplasmic antigen in people of different ages without known history of infection. Pediatrics 4-798.
Finlay, P., and Manwell, R. D.: 1956. Toxoplasma from the crow, a new natural host. Exper. Parasit. 5:149.
Frenkel, J. K.: 1956. Pathogenesis of toxoplasmosis and of infections with organisms resembling Toxoplasma. Ann. N.Y. Acad. Sci. 64.215.
Gang, D. P., and Manwell, R. D.: 1961. Some aspects of the cytochemical anatomy of *Toxoplasma gondii.* Jour. Parasit. 47:291.
Goldman, M., Carver, R. K., and Sulzer, A. J.: 1957. Similar internal morphology of *Toxoplasma gondii* and *Besnoitia jellisoni* stained with silver protein. Jour. Parasit. 43:490.

Goldman, M., Carver, R. K., and Sulzer, A. J.: 1958. Reproduction of *Toxoplasma gondii* by internal budding. Jour. Parasit. 44:161.

Guimarães, F. N., and Meyer, H.: 1942. Cultivo de "Toxoplasma" Nicolle and Manceaux, 1909, en culturas de tecidos. Rev. Brazil. Biol. 2:123.

Gustafson, P. V., Agar, H. D., and Cramer, D. I.: 1954. An electron microscope study of Toxoplasma. Am. Jour. Trop. Med. and Hyg. 3:1008.

Hepding, L.: 1939. Über Toxoplasma (*Toxoplasma gallinarum* n. sp.) in der Retina eines Huhnes and über deren Beziehung zur Huhnerlähmung. Zeitschr. f. Infektionskr. 55:109.

Herman, C. M.: 1937. Toxoplasma in North American birds and attempted transmission to canaries and chickens. Am. Jour. Hyg. 25:303.

——. 1938. The relative incidence of blood protozoa in some birds from Cape Cod. Trans. Am. Micr. Soc. 57:132.

Hoare, C. A.: 1956. Toxoplasmosis in animals. Vet. Rev. and Annot. 2:25.

Jacobs, L.: 1953. The biology of toxoplasma. Am. Jour. Trop. Med. and Hyg. 2:365.

——. 1956. Propagation, morphology, and biology of Toxoplasma. Ann. N.Y. Acad. Sci. 64:154.

——, and Jones, F. E.: 1950 The parasitema in experimental toxoplasmosis. Jour. Infect. Dis. 87.78

——, Melton, M. L., and Cook, M. K.: 1953. Experimental toxoplasmosis in pigeons. Exper. Parasit. 2:403.

——, Melton, M. L., and Jones, F. E.: 1952. The prevalence of toxoplasmosis in wild pigeons. Jour. Parasit. 38:457.

——, Remington, J. S., and Melton, M. L.: 1960. The resistance of the encysted forms of *Toxoplasma gondii*. Jour. Parasit. 46:11.

Johnson, C. M.: 1944 Immunological and epidemiological investigations. Gorgas Mem. Lab. Ann. Rep for 1943, p. 15

Kaufman, H. E., and Maloney, E. D.: 1962. Multiplication of three strains of *Toxoplasma gondii* in tissue culture. Jour. Parasit. 48:358.

Koestner, A., and Cole, C. R.: 1960. Neuropathology of canine toxoplasmosis Am. Jour. Vet. Res. 21:831

Levine, N. D.: 1961. Protozoan Parasites of Animals and Man. Burgess Publ. Co., Minneapolis, Minn. 412 pp.

Ludvik, J.: 1956. Vergleichende elektronenoptische Untersuchungen an *Toxoplasma gondii* und *Sarcocystis tenella*. Zentralbl. f. Bakt. I. Abt. Orig. 166:60.

Manwell, R. D., Coulston, F., Binckley, E. C., and Jones, V.: 1945. Mammalian and avian toxoplasma. Jour. Infect. Dis. 76:1.

——, and Drobeck, H. P.: 1951. Mammalian toxoplasmosis in birds. Exper. Parasit. 1:83.

——, and Drobeck, H. P.: 1953a. The behavior of Toxoplasma with notes on its taxonomic status. Jour. Parasit. 39:577.

——, and Drobeck, H. P.: 1953b Toxoplasmosis. Sci. Am. 188,86

Markham, F. S.: 1956. Part III. Toxoplasmosis. Introductory remarks. Ann. N.Y. Acad. Sci. 64 152

Nicolau, S.: 1932. Quelques propriétés d'un toxoplasme qui infecte spontanément les cobayes. Compt. Rend. Soc. Biol. 110:763.

Nobrega, P., and Reis, J.: 1942 Identidade dos toxoplasmas de aves e de mammiferos. Arq. Inst. Biol., São Paulo 13 21.

——, Trapp, E. E. and Giovannoni, M.: 1954. Toxoplasmosis en gallinas. Rev. Vet. Milit., Buenos Aires 2,209. (Jour Vet. Bull.—1955 Vol. 25, No. 1967.)

——, Trapp, E. E., and Giovannoni, M: 1955. Toxoplasmose espontânea de galinha. Arq. Inst. Biol., São Paulo 22:43

Pereira de Castro, M.: 1951. Divisão multipla de Toxoplasma em cultura de tecidos. Arq. Inst. Biol., São Paulo 22-233

Reis, J., Nobrega, P., and Reis, A. S.: 1936. Tratado de doenças das aves. Inst. Biol., São Paulo, 469 pp.

Rodhain, J.: 1950 Formation de pseudokystes au cours d'essais d'immunité croisée entre souches différentes de Toxoplasmes Compt Rend Soc Biol. 144:719.

——, and Gerebtzoff, M. A.: 1951 Au sujet de la membrane limitant les pseudokystes des toxoplasmes. Compt. Rend Soc. Biol 145-766.

Sabin, A. B.: 1939. Biological and immunological identity of toxoplasma of animal and human origin. Proc. Soc. Exper. Biol. and Med. 41:75.

——, and Feldman, H. A. 1948 Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoan parasite (Toxoplasma). Science 108:660.

——, and Olitsky, P. K.: 1937. Toxoplasma and obligate intracellular parasitism. Science 85:336.

Smn, J. C.: 1956. Toxoplasmosis acquared lymphonodosa: clinical and pathological aspects. Ann. N.Y. Acad. Sci. 64:183.

Springer, K.: 1942. Toxoplasmose enzootica entre pombos. (Lab. Paulista Biol., S. Paulo) Arq. Inst Biol., São Paulo 26 74 (Abst Biol. Abst. Vol. 16, (1942) Art. 23287).

Wenyon, C. M.: 1926. Protozoology. Ballière, Tindall, and Cox, London.

Westphal, A.: 1949. Über die Möglichkeit der Existenz und des serologischen Nachweises von Toxoalesminen. Zentralbl. f. Bakt. I Orig. 153.51.

————: 1954. Zur Systematik von *Toxoplasma gondii*. Zeitschr. f. Tropenmed. u. Parasit. 5:145.

————, and Palm, G.: 1953. Latente Toxoplasmainfektionen im Tierversuch als diagnostisches Hilfsmittel. I. Technik und Anwendung der Methode bei epidemiologischen Untersuchungen. Zeitschr. f. Tropenmedizin. u. Parasit. 4:322.

————, and Palm, G.: 1954. Latente Toxoplasmainfektionen im Tierversuch als diagnostisches Hilfsmittel. II. Anwendung der Methode bei klinischen Fällen und Untersuchungen zum mikroskopischen Parasitennachweis. Zeitschr. f. Tropenmedizin. u. Parasit. 5 61.

Wiktor, T. J.: 1950. Toxoplasmose animale. Sur une épidémie des lapins et des pigeons à Stanleyville (Congo Belge). Ann. Soc. Belge Med. Trop. 30:97.

Woke, P. A., Jacobs, L., Jones, F. E., and Melton, M 1953. Experimental results on possible arthropod transmission of toxoplasmosis. Jour. Parasit. 39:523.

Wolf, A., Cowen, D., and Paige, B.: 1939. Human toxoplasmosis: occurrence in infants as an encephalomyelitis verification by transmission to animals. Science 89:226.

Wolfson, F.: 1940. Organism described as avian *Toxoplasma*. Am. Jour. Hyg. 32.88, Sec. C.

————: 1941. Mammalian *Toxoplasma* in erythrocytes of canaries, ducks, and duck embryos. Am. Jour. Trop. Med, 21:653.

HAEMOPROTEUS INFECTIONS

The genus Haemoproteus belongs to the family Haemoproteidae which is fundamentally like the family Plasmodiidae, to which the true malaria parasites belong, except that schizogony occurs in endothelial cells of internal organs rather than in circulating blood cells. Ordinarily it is impossible to transfer the infection by inoculation of infected blood, as can be done with the true malarias. (Cf. Lastra and Coatney, 1950.) Haemoproteus differs from Leucocytozoon in two important respects: (1) while in both genera the mature gametocytes are the stages generally occurring in the circulating blood, those of Haemoproteus occupy erythrocytes and those of Leucocytozoon, supposedly, only white cells, such as monocytes or macrophages; (2) true malarial pigment is produced in the gametocytes of Haemoproteus, while the pigmentlike granules of Leucocytozoon, if any, disappear from specimens on stained slides after washing in dilute acetic acid and ammonia. The life cycle in general parallels that of Plasmodium and Leucocytozoon, but the intermediate hosts of Haemoproteus species are usually hippoboscid flies, commonly called louse flies, though Fallis and Wood (1957) have shown that an orthorrhaphous insect, a biting midge, *Culicoides* sp., is a suitable intermediate host and transmitting agent of *H. nettionis* of ducks. Coatney (1936) published a check list and host index of the genus Haemoproteus in which appear 45 specific names, most of which are described from birds. The genus occurs widely in passerine birds, owls, flickers, woodpeckers, ducks, and other types of birds, and in certain reptiles as well. Herman (1938a) found 50 per cent of the chipping sparrows on Cape Cod infected.

Haemoproteus lophortyx O'Roke, 1929, is a pathogenic parasite of California valley quail (see O'Roke, 1930, and Herman and Glading, 1942). It is doubtful if this bird ever completely recovers once it has become infected (Herman and Bischoff, 1949). The vectors are louse flies *Lynchia hirsuta* (cf. O'Roke, 1930) and *Stilbometopa impressa* (cf. Herman and Bischoff, 1949).

Other species of quail have also been found infected, as follows: in California, San Quentin quail, Cambel's quail, and mountain quail (see Wood and Herman, 1943); in New Mexico and Arizona, Cambel's quail and scaled quail; in District of Columbia and vicinity, the bobwhite (Campbell and Lee, 1953; Hungerford, 1955).

H. nettionis Cleland and Johnston, 1909, has for its type host the Australian teal, *Anas* (= *Nettion*) *castaneum*. Herman (1954), in a critical review, suggests that this is the correct name for the *Haemoproteus* of Anatidae, and assigns *H. anatis* Haiba, 1946, and *H. hermani* Haiba, 1948, to synonymy. The gametocytes do not affect the size of infected cell, but may push the nucleus aside. The following are some of the other known hosts: Indian runner duck, common tame duck (White Pekin) (Chernin and Sadun, 1949), white

Chinese goose, Canada goose, whistling swan, mallard, black duck, green-winged teal, blue-winged teal, cotton tast, shoveller, American pintal, wood duck, baldpate, common goldeneye, ring-necked duck, and common merganser. (See Herman, 1951, 1951; Wood and Herman, 1913; Wetmore, 1911; Levine and Hanson, 1953; Fallis and Wood, 1957.) The vector in certain parts of North America is probably a species of *Culicoides*, since Fallis and Wood have shown that biting midges of this genus occur in areas where the parasite occurs in ducks, will feed on ducks, are suitable hosts for the parasite, and, when infected specimens are ground up and injected into clean ducks, carry the sporozoites capable of producing the infection with an incubation period of 14 to 21 days. The infection appears to be well tolerated by its hosts.

H. danilewskyi Kruse, 1890, whose type host is the gray crow and has been found in various types of birds, was merely recorded as occurring in the Red Jungle Fowl of Malay (*Gallus gallus*) by Plimmer (1913). The bird had been kept in a London zoo.

H columbae Kruse, 1890, from the common pigeon (Fig. 37.7) and *H. maccallumi* Novy and MacNeal, 1905, from the mourning dove seem to be morphologically identical. Besides, the parasite from both hosts has been transferred to the pigeon by means of the blood-sucking intermediate host, *Pseudolynchia canariensis* (= *Lynchia maura* = *P. maura*) by Huff (1932). Since it was the first of the genus to have its life cycle in the pigeon and louse fly completely outlined, accounts of it appear in standard works on protozoology such as Wenyon (1926) and Kudo (1954). *Microlynchia pusilla* in South America and *Ornithomyia avicularia* in England are two other louse flies suspected of being vectors (cf. Baker, 1957). Although schizogony does not take place in the peripheral blood, Lastra and Coatney (1950) showed that it is possible to transfer the infection by direct injection of considerable quantities of blood or transplan-
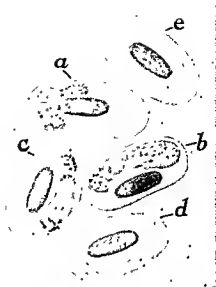
FIG. 37.7 — **Haemoproteus columbae.** Pigeon blood. a, b—macrogametocyte in erythrocyte. c—microgametocyte. d, e—normal erythrocyte. (Drake and Jones.)

tation of tissues from donor pigeons taken early in the course of its infection. The infection occurs in pigeons widely throughout the tropical and subtropical regions and in the temperate zones wherever the insect vector can survive, but only sporadically in a climate such as prevails in Iowa where the vector ordinarily dies out in the winter if introduced by chance during the spring or summer (Drake and Jones, 1930). In England it occurs in wood pigeons (Baker 1957). Becker (1959) reported that he frequently found gametocytes of *H. columbae* in the blood of adult and nestling mourning doves in Iowa, but never observed louse flies on these birds (cf. Hanson *et al.*, 1957, in Illinois). Wood and Herman (1913) record it in the western mourning dove, western white-winged dove, and band-tailed pigeon. Levine and Kantor (1959) tabulated reports of *Haemoproteus*, judged to be *H. columbae*, from 45 species of columbiform birds. This parasite is not a severe pathogen, although Coatney (1933) observed one bird with a heavy infection whose behavior was for a while definitely abnormal.

H. sacharovi Novy and MacNeal, 1904.

is one of the numerous species of Haemoproteus, described from various wild birds, that should be mentioned because, while normally a parasite of mourning doves, it occurs at times in the common pigeon (see Coatney and West, 1940; Becker *et al.*, 1956). Other known hosts are the western mourning dove and the western white-winged dove. The oval or round gametocytes, unlike those of most other species of Haemoproteus, enlarge their host cells, erythrocytes, about 1.3 times in width and 1.4 times in length, and push the nucleus to one side, although it sometimes surrounds it. The amount of pigment is small. The known bird hosts are the eastern mourning dove, the western mourning dove, and the western white-winged dove (see Wood and Herman, 1943; Herman, 1944). Huff (1932) transferred this species from the dove to a pigeon using the louse fly, *Pseudolynchia canariensis*, for the vector. However, as he points out in a later publication (1963), "demonstration of one means of transmission has often been followed by the *only* means," and he then cites instances to show how such assumptions may retard research. As Levine (1961) observes, the natural vector of *H. sacharovi* is unknown; the *Culicoides* is a possibility.

Haemoproteus meleagridis Levine, 1961. Wetmore, 1941, seems to have been the first to record Haemoproteus as a parasite of domestic turkeys (from District of Columbia and vicinity). Morehouse (1945)

next observed the gametocytes of Haemoproteus in the blood of a turkey sent from Texas. The infection was a heavy one in an anemic bird from a flock that "seemed to have a good appetite, but just wasted away." The parasite was also observed by Kozicky (1948) in a native wild gobbler and four wild turkey hens reared in captivity—a total of five out of 97 blood smears of wild turkeys collected in 1947. Goldsby (1951) reported the occurrence of Haemoproteus in a flock of turkeys in North Dakota. Three out of ten turkeys examined in South Carolina by Atchley (1951) harbored the parasite. Bierer *et al.* (1959), also working in South Carolina, found a "Haemoproteuslike" organism in 22 of 52 domestic turkeys. Two of the birds were heavily parasitized, and were obviously ill. These workers suggested that wild turkeys might have been the source of infection. Love *et al.* (1953) found Haemoproteus in 1 of 2 wild turkeys taken in Georgia. Morehouse's figures indicate little or no enlargement of the infected erythrocyte, and the position of the cell nucleus seems to be altered in some but not in others.

Treatment. Atabrin and pamakin (plasmochin), according to Coatney (1935), affect *Haemoproteus columbae* infection. Atabrin inhibits the development of young gametocytes, while pamakin (plasmochin) does not. The latter is parasiticidal to adult gametocytes, however; neither seems to affect the schizonts.

REFERENCES

Atchley, F. O.: 1951. *Leucocytozoon andrewsi* n. sp., from chickens observed in a survey of blood parasites of domestic animals in South Carolina. Jour. Parasit. 37:483.
Baker, J. R.: 1957. A new vector of *Haemoproteus columbae* in England. Jour. Protozool. 4:204.
Becker, E. R.: 1959. Protozoa, Chapter 36, in Biester and Schwarte, Diseases of Poultry. 4th ed. Iowa State Univ. Press, Ames, Iowa. P. 890.
——— Hollander, W. F., and PattiBo, W. H.: 1956. Naturally occurring Plasmodium and Haemoproteus infection in the common pigeon. Jour. Parasit. 42.474.
Bierer, B. W., Vickers, C. L., and Thomas, J. B.: 1959. A parasitism in turkeys due to a Haemoproteus-like blood parasite. Jour. Am. Vet. Med. Assn. 135:181.
Campbell, H., and Lee, L.: 1953. Studies on quail malaria in New Mexico and notes on other aspects of quail populations. New Mexico Dept. of Game and Fish, Santa Fe, N.M. 79 pp.
Chernin, E., and Sadun, E. H.: 1949. *Leucocytozoon simondi* infections in domestic ducks in northern Michigan with a note on Haemoproteus. Poultry Sci. 28:890.
Coatney, G. R.: 1935. Relapse and associated phenomena in the Haemoproteus infection of the pigeon. Am. Jour. Hyg. 18:133.

Coatney, G. R.: 1935. The effect of atabrine and plasmochin on the Haemoproteus infection of the pigeon. Am. Jour. Hyg. 21:249.

———: 1936. A check list and host-index of the genus Haemoproteus. Jour. Parasit. 22:88.

———, and West, E.: 1940. Studies on *Haemoproteus sacharovi* of mourning doves and pigeons, with notes on *H. maccallumi*. Am. Jour. Hyg. 31:9, Sec. C.

Drake, C. J., and Jones, R. M.: 1930. The pigeon fly and pigeon malaria in Iowa. Iowa St. Coll. Jour. Sci. 4:253.

Fallis, A.M., and Wood, D. M.: 1957. Biting midges (Diptera: Ceratopogonidae) as intermediate hosts for Haemoproteus of ducks. Canad. Jour. Zool. 35.425.

Goldsby, A.I.: 1951. Poultry parasites new to North Dakota. Bi-m Bul. No. Dakota Agr. Exper. Sta. 13:121.

Hanson, H. C., Levine, N. D., Kossack, C. W., Kantor, S., and Stannard, L. J.: 1957. Parasites of the mourning dove (*Zenaidura macroura carolinensis*) in Illinois. Jour. Parasit. 43:186.

Herman, C. M.: 1938a. The relative incidence of blood protozoa in some birds from Cape Cod. Trans. Am. Micr. Soc. 57:132.

———: 1938b. Haemoproteus sp. from the common black duck, *Anas rubripes tristis*. Jour. Parasit. 24:53.

———: 1944. The blood protozoa of North American birds. Bird Banding 15:89.

———: 1945. Hippoboscid flies as parasites of game animals in California. Calif. Fish and Game 31:16.

———: 1951. Blood parasites from California ducks and geese. Jour. Parasit. 37:280.

———: 1954 Haemoproteus infections in waterfowl. Proc. Helm. Soc. Washington 21:37.

———, and Bischoff, A. I.: 1939. The duration of Haemoproteus infection in California quail. Calif. Fish and Game 35:293.

———, and Glading, B.: 1942. The protozoan blood parasite *Haemoproteus lophortyx* Clark in quail at the San Joaquin Experimental Range, California. Calif. Fish and Game 28:150.

Huff, C. G.: 1932. Studies on Haemoproteus of mourning doves. Am. Jour. Hyg. 16:618.

———: 1942 Schizogony and gametocyte development in *Leucocytozoon simondi* and comparisons with Plasmodium and Haemoproteus. Jour. Infect. Dis. 71:18.

———: 1963 Experimental research on avian malaria. Advances in Parasitology. Academic Press, New York. 1-1.

Hungerford, C. R 1955 A preliminary evaluation of quail malaria in southern Arizona in relation to habitat and quail mortality. Trans. Twentieth No. Am. Wildlife Conf. 209 pp.

Koutz, E. J.: 1948. Some protozoan parasites of the eastern wild turkey in Pennsylvania. Jour. Wildlife Mgt. 12:263

Kudo, R. R. 1951 Protozoology, 4th ed. Charles C Thomas, Springfield, Ill.

Laura, I., and Coatney, G. R.: 1940 Transmission of *Haemoproteus columbae* by blood inoculation and tissue transplants. Jour. Nat. Malaria Soc. 9:151

Levine, N. D.: 1961 Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn. 412 pp.

———, and Hanson, H. C.: 1953. Blood parasites of the Canada goose. Jour. Wildlife Mgt. 17:185

———, and Kantor, S.: 1959. Checklist of blood parasites of the order Columbiformes. Wildlife Dis 1 1. (Microcard)

Love, G. J., Wilkin, S A., and Goodwin, M. H., Jr.: 1953. Incidence of blood parasites in birds collected in southwestern Georgia. Jour. Parasit. 39:52.

Morehouse, N F 1945 The occurrence of Haemoproteus sp. in the domesticated turkey. Trans. Am. Micr. Soc. 64:109

O'Roke, E. C.: 1929. The morphology of *Haemoproteus lophortyx* sp. new. Science 70:432.

———: 1930 The morphology, transmission, and life-history of *Haemoproteus lophortyx* O'Roke, a blood parasite of the California valley quail. Univ. Calif. Publ. Zool. 36:1.

———: 1932. Parasitism of the California Valley quail by *Haemoproteus lophortyx*, a protozoan blood parasite. Calif. Fish and Game 18:223.

Plummer, F. G. 1913 Report on deaths which occurred in the zoological garden during 1912..., together with blood parasites found during the year. Proc. Zool. Soc. London 1913 (1), Mar., p. 141.

Reis, J., and Nobrega, P.: 1956. Tratado de Doencas das Aves. São Paulo, Brazil.

Wenyon, C. M.: 1926. Protozoology. Baillière, Tindall, and Cox, London.

Wetmore, P. W.: 1941. Blood parasites of birds of the District of Columbia and Patuxent Research Refuge vicinity. Jour. Parasit. 27:379.

Wood, S. F., and Herman, C. M.: 1943. The occurrence of blood parasites in birds from southwestern United States. Jour. Parasit. 29 187.

PLASMODIUM INFECTIONS

The true malarial organisms belong to the genus Plasmodium, which in turn is closely related to Haemoproteus and Leu- cocytozoon. The principal significant difference between Plasmodium and the other two genera is that the asexual stages (schizonts) of the former occur in erythro-

cytes of the circulating blood, while those of the two latter occur in the internal organs (lung, liver, spleen, kidney, etc.). As a result, Plasmodium can be transmitted regularly from one susceptible host to another by injection of infected blood from the vessels or heart, while in the case of the other two this procedure will result in infection only at certain times when merozoites are in the blood by chance, because the only stages ordinarily in the blood are gametocytes which continue development in the proper invertebrate host. Like Haemoproteus, Plasmodium contains pigment. The life cycle, as is well known, involves two hosts: an intermediate host, a vertebrate, in which asexual multiplication (schizogony) and the formation of immature sexual forms (gametocytes) occur, and a definitive host, presumably always a mosquito, in which maturation of the gametes, fertilization, and sporogony take place. It is of special interest that mammalian malarias are carried by Anopheles mosquitoes, while those of birds are carried by culicine (Culex, Aedes) mosquitoes, although some of the latter have also anopheline vectors.

The story of the development of Plasmodium in vertebrates from sporozoite, the stage introduced by the mosquito while biting, to the earliest stages observed in erythrocytes, was the contribution resulting from the brilliant researches of a number of investigators, among them Huff and Coulston (1944). The sporozoite of *P. gallinaceum* enters a "lymphoid macrophage" cell to develop in about 42 hours into a "cryptozoite," or a schizont undergoing schizogony. The merozoites of this generation enter other similar cells to repeat schizogony in another 40 hours (the "metacryptozoites"). Blood infections then ensue, presumably from the merozoites from the fixed tissue stages. Subsequent development of Plasmodium within the vertebrate host usually follows the general course outlined below.

The parasite as first seen in the red blood cells in films treated with a Romanowsky stain may have the appearance of a signet ring. The center of the parasite is occupied by a vacuole, which does not stain. The surrounding cytoplasm, which stains blue, therefore appears as a ring. The nucleus, also located peripherally, stains red. The signet ring effect is observed when this stage, now called a trophozoite, is viewed at right angles to the plane containing the nucleus. The trophozoite grows at the expense of the red cell, actually engulfing hemoglobin which, when digested, leaves a residue of pigment. Eventually the chromatin material divides, followed by division of the cytoplasm, and the segmenting schizont gives rise to merozoites. The number of merozoites formed depends upon the species, as does the time required for their development, at least under fixed conditions. The schizonts of different cells usually mature at about the same time, so large numbers of red blood cells may be destroyed in a short time, resulting in the liberation of toxic products, as well as merozoites. The merozoites so liberated now enter other red cells, and repeat the asexual cycle until some develop into sexual forms, microgametes and macrogametes, also within red blood cells. These sexual cells undergo no further development until taken up by a mosquito. The gametes and the schizonts, both in the peripheral blood, may be sufficiently characteristic for a given species of Plasmodium to make specific identification possible.

More than 30 species of Plasmodium have been described from birds of various kinds (see Hewitt, 1940), but fewer than half are presently regarded as valid (see Levine, 1961; Bray, 1957; Laird and Lari, 1958). The volume of literature on avian malaria is immense, many of the contributions having dealt with drug screening and other studies related to malaricidal properties of various drugs. There are also a great many contributions on other aspects of experimental research, such as studies on immunity, genetics, *in vitro* cultivation, interrelationship with other infections, and basic physiology and biochemistry. A brief but excellent review of representative contributions to these and other fields of ex-

perimental research on avian malaria has been presented by Huff (1963). He was, however, obliged to restrict his coverage largely to the period 1955–1962. Nevertheless, the papers cited by Huff, together with the references cited therein, will lead the interested investigator to the literature on almost any aspect of avian malaria with little difficulty.

The following more or less general papers and their bibliographies are also commended to the interested reader: Coatney and Roudabush (1937), Coatney and West (1938), Herman (1938, 1944), Herman et al. (1954), Hewitt (1940), Huff (1932, 1935a, 1939), Kikuth (1931), Levine and Hanson (1953), Manwell (1935, 1938), Sergent et al. (1931), and Wolfson (1941).

Most of the avian species of Plasmodium are far less host specific than are some of the better known mammalian forms. Some occur naturally in a considerable number of species of wild birds, and some have been adapted by experimental passage to develop in birds in which they are not known to occur naturally. Only a few species have been reported as responsible for natural infections in domestic birds, and it is by no means certain that all of these are of veterinary importance. Some are at least of potential importance, and these species will be discussed briefly.

Before doing so, however, it should be noted that naturally occurring Plasmodium infections have been found in chickens in the United States for the first time (Krishnamurti et al., 1962), and that the organism did not correspond exactly to any thus far reported as occurring naturally in the domestic fowl. The authors compare their organism to P. gallinaceum, but do not identify it specifically as such.

Plasmodium gallinaceum Brumpt, 1935. The British veterinarian M. Crawford, while stationed in Ceylon, announced in the official 1933 report to his government that cases of bird malaria had developed on that island in chickens recently imported from England, and he described the symptoms and advocated quinine for treatment. He assigned the microorganism to "Plasmodium praecox." Later, Crawford (1915) carefully narrated the history of the parasite and the manner in which it became available as a satisfactory laboratory subject in the war program for obtaining new and better antimalarial drugs. Also, he acknowledged the correct name to be P. gallinaceum, described by Brumpt in 1935 from a blood smear presented to him in 1910 by a Dr. Broussais who had seen the malarial organism in fowls in Indo-China. Brumpt stated that from 1935 to 1948 more than 600 publications on P. gallinaceum had enriched practical and theoretical knowledge concerning malaria, and many more papers have appeared since 1948.

Crawford considered jungle fowls to be the natural hosts—Gallus lafayetti in Ceylon, G. bankiva in India, and G. sonnerati in Sumatra. (Brumpt, however, believed the native host to be a still undetected wild bird.) These native fowl (Gallus) are quite resistant to the infection, but when imported breeds are introduced into areas where the wild fowl are infected, they suffer intense infections which generally lead to death after a brief illness. Birds may at times develop paralysis and die after drug treatment owing to blocking of brain capillaries with the large nonpigmented exoerythrocytic stages of the parasite discovered by James and Tate (1937, 1938). Because he believed the chicken not susceptible to known avian malarias (see, however, Manwell, 1933), Brumpt felt confident he was dealing with a new species. Ducks, guinea fowl, pigeons, turtle doves, quail, buzzards, canaries, sparrows, calfats, and finches were later shown by him to be resistant to P. gallinaceum, while chickens of various breeds, geese, pheasants, partridges, and peacocks were susceptible. The infection assumed a more acute form in young chicks and a more chronic course in the adult birds (see also Coggeshall, 1938).

Since Crawford's (1915) summary, Haiba (1948) reported the parasite in the blood of a dead chicken in Egypt, but its occurrence in that country requires con-
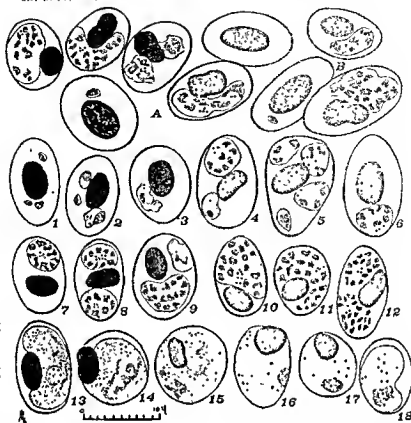
FIG. 37.8 — Plasmodium gallinaceum. A and B—group of erythrocytes showing alterations in the chromatin of the infected cells. 1—12—simple or multiple infections of the erythrocytes by trophozoites and schizonts in different stages of development; 13, 14, 10, 11, and 12 represent the formation of merozoites; 13, 14, and 15, male gametocytes; 16, 17, and 18, female gametocytes; 18, female gametocyte in an enucleated erythrocyte. (Note: The solid black bodies are the nuclei of the erythrocytes.) (After Brumpt, 1935.)

firmation. Occurrence of two naturally acquired infections in exotic fowl at the Izatnagar (India) Poultry Farm was first noted by Rao et al. (1951), and later Das et al. (1952). The symptoms, postmortem lesions and histopathology observed in fowls infected by direct blood inoculation are described in considerable detail. Kranceveld and Mansjoer (1953) found the parasite in West Java, and submitted authority for its occurrence also in Sumatra, Java, and the Celebes.

Beltran (1941a) published a summary of the state of our knowledge concerning this species up to that year, but much information has been added since. Jacobi (1939) has studied the pathology of P. gallinaceum infection. The discovery of an exoecythrocytic phase, i.e., an asexual developmental cycle in endothelial cells or reticuloendothelial cells of spleen, brain, liver, etc., by James and Tate (1938) and James (1939), has attracted a great deal of attention, because it was formerly believed that the increment of malaria parasites in the vertebrate host occurred entirely within erythrocytes. For morphological and other details the reader should consult Brumpt's papers. The exoerythrocytic stages preceding the erythrocytic stages described by Huff and Coulston (1944) have been mentioned above.

The natural vectors, of course, are not known, but some prominently mentioned potential mosquito vectors are *Aedes aegypti, A. albopictus, A. geniculatus,* and *Culex quinquefasciatus* (see Brumpt, 1936a, b; Vargas and Beltran, 1941). Huff (1954) has listed 29 susceptible species and one questionable belonging to various genera as follows: Anopheles, 1; Culiseta, 1; Mansonia, 1; Aedes, 19; Armigeres, 5; Culex, 2 proved, 1 questionable (*quinquefasciatus*). Beltran and Larenas (1911) have also demonstrated its transmission by the oral route.

Plasmodium juxtanucleare Versiani and Furtado Gomes, 1941, occurs chiefly in chickens. It has been recorded only from South America and from the State of Chiapas, Mexico (Beltran, 1941b). The parasites tend to lie in contact with the host cell nucleus and distort the cell. There are about four merozoites. The cycle is about 24 hours, and this parasite is said to be a severe pathogen. It does not grow in canaries.

Plasmodium durae Herman, 1941. This species was found in a blood smear of one out of 75 domestic turkeys examined in Kenya Colony, British East Africa It was capable of afflicting young turkeys fatally.

Plasmodium cathemerium Hartman, 1927, the type host of which is the English sparrow, resembles *P. relictum* in that it occurs commonly in passerine birds, causes the nucleus of the infected cell to be displaced more or less toward one pole or expelled, and has roundish gametocytes; but it differs from that species in that the pigment grains in the gametocytes are relatively coarse and rodlike. An outbreak of *P. cathemerium* malaria in California that cost a canary raiser possibly 165 out of 700 of these birds was reported by Mathey (1955b). The sick birds exhibited swelling in the region of the eyes. The characteristic parasites were found in blood smears. Necropsy disclosed subcutaneous hemorrhage, splenomegaly and hepatomegaly. Sick birds responded to atabrin. Herman and Vail (1942) reported

a fatal case of spontaneous malaria in a canary from Temple City, California. Hewitt (1939) made a special study of splenic enlargement and infarction in infected canaries.

Plasmodium relictum Crassi and Feletti, 1891. *P. relictum,* of which *P. praecox* is a synonym, is a species occurring in many species of wild birds, among them the eastern (Coatney, 1938) and western mourning doves (Herman *et al.,* 1954) and certain wild waterfowl such as the pintail, cinnamon teal (Herman, 1951), wood duck (Mielcarek, 1954), and American coot (Roudabush, 1912). Coatney (1938) obtained identical strains from the wild mourning dove and common pigeon. The strain was extremely pathogenic in pigeons, but in doves and canaries the infections were light and transitory. When transferred to chicks, the infections lasted 6 to 11 days. The first observers of naturally occurring *P. relictum* in pigeons, however, were Sergent and Sergent (1904), working in Algeria. Others were as follows: Pelaez *et al.* (1951), in Mexico; Mathey (1955a), in California; and Becker *et al.* (1956), in Iowa. Mathey observed three infected pigeons, at least two of which succumbed to malaria. He also succeeded in infecting the chick and the canary with parasites from the pigeon. This species resembles *P. cathemerium* morphologically, but its pigment grains are roundish instead of elongate. Some strains of it, like *P. cathemerium,* have diurnal periodicity. The infection responds readily to treatment with quinine or atabrin. Wolfson (1938) also obtained infections in ducks with two other strains of *P. relictum.* Hill (1942) has proved rather conclusively that anemia may be regarded as the cause of death of pigeons in *P. relictum* infections.

Plasmodium circumflexum Kikuth, 1931, has for its type host a German thrush (*Turdus pilaris*). Its morphological characteristics are as follows: all stages occurring in the circulating blood; elongate gametocytes; from 13 to 30 merozoites produced per schizont; and both schizonts and

gametocytes tending to encircle the nucleus of the infected cell without displacing it. A Plasmodium morphologically similar in many respects to this one was observed by Fallis (1945, 1946) in ruffed grouse in Ontario, Canada. It developed in canaries, but not so readily as in grouse, while a canary strain would not develop in grouse. The grouse strain is not pathogenic in grouse, canaries, or ducks, and would not develop in chickens or pheasants.

Plasmodium lophurae Coggeshall, 1938. This species was isolated by Coggeshall (1938) from a Borneo fireback pheasant, *Lophura igniti igniti*, at the New York Zoological Park. It is transmissible to very young chicks, but as a rule produces a moderately severe attack that does not terminate fatally. Only mild infections may be produced in adult fowls, and canaries are not susceptible. The original description should be consulted for morphological and other details. Terzian (1941a and b) has recently made an excellent study of the biological characteristics, pathology, and effects of this interesting species in chicks. Laird (1941) showed that *P. lophurae* can be transmitted from duck to duck through the agency of the mosquito *Aedes albopictus*, and he succeeded in infecting also *Culex restuans* and *Aedes atropalpus*. *Anopheles quadrimaculatus* can also be infected, at least lightly (cf. Coggeshall, 1941, and Hurlbut and Hewitt, 1941, 1942). Trager (1942) obtained by selective breeding a strain of *Aedes aegypti* which was more susceptible to *P. lophurae* than the stock from which it had its origin.

Severe infections with high parasitemias are produced in ducks (see Wolfson, 1941). Hewitt's (1942) study of host-

parasite relationship of *P. lophurae* infection in ducks, with its excellent colored plates of the parasite and types of blood cells affected by the infection, is especially commended to the reader's attention. The course of unrelated blood-induced infections in 1,200 young White Pekin ducks was charted by Hewitt et al. (1942). Becker *et al.* (1949) studied the course of infection in some of the comparatively few ducks that had survived the primary attack, and found that in only one of 26 did the infection become permanently latent, while in all the rest it followed a relapsing course with subpatent periods of varying length alternating with patent periods of varying length and with parasitemias of varying intensity. Ducks exhibit reverse age resistance to this parasite in contrast to chicks, which become more resistant as they grow older (Becker, 1950).

Exoerythrocytic forms (phanerozoites) have never been detected in chickens or ducks, although Becker and Manresa (1950) and Manresa (1953) located them in brain capillaries in turkey infections. As in *P. gallinaceum*, they may cause the death of the host by blocking the capillaries, even though the parasitemia is low. Stauber and van Dyke (1945) have compared *P. cathemerium* and *P. lophurae* infections in duck embryos. Goslings are highly susceptible (Becker, 1951). This species, like *P. gallinaceum*, has served as an excellent subject for antimalarial investigations.

For information on domesticated birds as experimental hosts of avian plasmodia, the reader is referred to Wolfson (1941), Manwell (1933, 1943, 1952), and Huff (1963).

REFERENCES

Atchley, F. O.: 1950. A survey of blood parasites in domestic animals in South Carolina. Jour. Parasit. 36 (Suppl.):22.

Becker, E.R.: 1950. Mortality in relation to age in young White Pekin ducks with blood-induced *Plasmodium lophurae* infection. Proc. Iowa Acad. Sci. 57:435.

——: 1951. The course of blood-induced *P. lophurae* malaria in young goslings and guinea fowl chicks. Jour. Parasit. 37 (No. 5, Sec. 2):12.

——, Brodine, C. E., and Clappison, B. L.: 1949. The post-crisis in blood-induced *Plasmodium lophurae* infections in White Pekin ducks. Iowa St. Univ. Jour. Sci. 23:237.

Becker, E. R., Hollander, W. F., and Pattillo, W. H   1956 Naturally occurring Plasmodium and
    Haemoproteus infection in the common pigeon. Jour. Parasit. 42 474.
————, and Manresa, M., Jr: 1950. Phanerozoites in turkeys succumbing with blood-induced
    Plasmodium lophurae infection. Iowa St. Univ. Jour. Sci. 25:353.
Beltran, E: 1941a. Estado actual de nuestros conocimientos acerca del Plasmodium gallinaceum
    Brumpt 1935. Rev. del Inst. Salubr. y Enferm. Trop. 2.95.
————: 1941b. Hallazgo de Plasmodium juxtanucleare Versiani y Furtado en gallinas de Chiapas.
    Rev. del Inst. Salubr. y Enferm. Trop. 2:353.
————, and Larenas, M. R: 1941. Produccíon de malaria avair con Plasmodium gallinaceum por
    vía oral. Rev. del Inst. Salubr. y Enferm. Trop. 2.97.
Bray, R. S.: 1957. Studies on the exoerythrocytic cycle in the genus Plasmodium. London Sch.
    Hyg Trop. Med. Mem, 12, H. K. Lewis, London.
Brumpt, E: 1935. Paludism avianer Plasmodium gallinaceum, n. sp. de la poule domestique.
    Compt. Rend. Acad. Sci. 200:783.
————: 1936a Réceptivité de divers oiseaux domestiques et sauvages au parasite (Plasmodium
    gallinaceum) du paludisme de la poule domestique. Transmission de cet hématozoaire par le
    moustique Stegomyia fasciata. Compt Rend. Acad Sci 203.750.
————: 1936b. Etude expérimentale du Plasmodium gallinaceum, parasite de la poule domes-
    tique. Transmission de ce germe par Stegomyia fasciata et Stegomyia albopicta. Ann. Parasit.
    Hum et Comp. 14:597.
Coatney, G R. 1938. A strain of Plasmodium relictum from doves and pigeons infective to
    canaries and the common fowl, Am. Jour Hyg. 27 390.
————, and Roudabush, R I. 1937 Some blood parasites from Nebraska birds. Amer. Midl.
    Nat 18:1003
————, and West, E. 1938. Some blood parasites from Nebraska birds. II Am. Midl. Nat. 19.601.
Coggeshall, L T . 1938. Plasmodium lophurae, a new species of malaria parasite pathogenic for
    the domestic fowl. Am. Jour. Hyg. 27.615.
———— 1941 Infection of Anopheles quadrimaculatus with Plasmodium cynomolgi, a monkey
    malaria parasite, and with Plasmodium lophurae, an avian malarial parasite. Am. Jour,
    Trop. Med. 21:525.
Crawford, M : 1945. Plasmodium gallinaceum, a malatial parasite of the domestic fowl. Vet.
    Record 57:395.
Das, J, Rao, S B. V., and Ramnant, D R.: 1952 Studies on Plasmodium gallinaceum. Indian
    Vet. Jour. 29 14
Eyles, D. E.: 1951 Studies on Plasmodium gallinaceum. I Am Jour Hyg. 51:101.
————: 1952a. Studies on Plasmodium gallinaceum. II. Am Jour. Hyg 55:276.
————: 1952b Studies on Plasmodium gallinaceum. III. Am. Jour. Hyg. 55:386.
————: 1952c. Studies on Plasmodium gallinaceum. IV. Am. Jour. Hyg. 56.71.
Falls, A. M: 1945 Population trends and blood parasites of ruffed grouse in Ontario. Jour.
    Wildlife Mgt. 9:203.
———— 1946. Plasmodium circumflexum (Kikuth) in ruffed grouse in Ontario. Jour. Parasit.
    32:345.
Haiba, M. H.: 1948. Plasmodia of common Egyptian birds. Jour. Comp, Path. 58.81.
Herman, C. M.: 1938. The relative incidence of blood protozoa in some birds from Cape Cod,
    Trans. Am. Micr. Soc. 57:132.
———— 1941. Plasmodium durae, a new species of malaria parasite from the common turkey.
    Am. Jour. Hyg. 34:22, Sec. C
———— 1944. The blood protozoa of North American birds. Bird banding 15.89.
———— 1951. Blood parasites from California ducks and geese. Jour. Parasit. 37:280.
————, Reeves, W. C, McClure, H. E., French, E. M., and Hammon, W. McD.: 1954. Studies
    on avian malaria in vectors and hosts of encephalitis in Kern County, California. Am. Jour.
    Trop. Med and Hyg. 3.676.
————, and Vail, E. L.: 1942. A fatal case of spontaneous malaria in a canary. Jour. Am. Vet.
    Med. Assn. 101.502.
Hewitt, R: 1939. Splenic enlargement and infarction in canaries infected with a virulent strain
    of Plasmodium cathemerium. Am. Jour. Hyg. 29:829, Sec. C.
———— 1940. Bird malaria Am. Jour. Hyg. Mon. Series, No. 15, The Johns Hopkins Press,
    Baltimore.
———— 1942. Studies on the host-parasite relationships of untreated infections with Plas-
    modium lophurae in ducks. Am. Jour. Hyg. 36.6.
————, Richardson, A. P., and Seager, L. D: 1942. Observations on untreated infections with
    Plasmodium lophurae in twelve laughing gulls. Am. Jour. Hyg. 36.173.
Hill, C, McD: 1942. Anemia as a cause of death in bird malaria. Am. Jour. Hyg. 36:562.
Huff, C. G: 1932. Further infectivity experiments with mosquitoes and bird malaria. Am. Jour.
    Hyg. 15:751.
———— 1935a. Natural immunity and susceptibility of culicine mosquitoes to avian malaria. Am.
    Jour. Trop. Med. 15:427.
————: 1935b. Plasmodium hexamerium, n sp, from the bluebird, inoculable to canaries. Am.
    Jour. Hyg. 22:274.
———— 1939. A survey of blood parasites of birds caught for banding purposes. Jour. Am. Vet.
    Med. Assn. 94:615.

_____: 1954. A review of the literature on susceptibility of mosquitoes to avian malaria, with some unpublished data on the subject. Res. Rep. Naval Med. Res. Inst. Vol. 12, Dec. 17, 1954.

_____: 1963. Experimental research on avian malaria. Advances in Parasitology, 1:1. Academic Press, London and N.Y.

_____, and Bloom, W.: 1935. A malarial parasite infecting all blood and blood-forming cells of birds. Jour. Infect. Dis. 57:315.

_____, and Coulston, F.: 1944. The development of Plasmodium gallinaceum from sporozoite to erythrocytic trophozoite. Jour. Infect. Dis. 75:231.

Huriburt, H. S., and Hewitt, R.: 1941. Sporozoites of Plasmodium lophurae, an avian malaria parasite, in Anopheles quadrimaculatus. Pub. Health Reps. 56:1336.

_____, and Hewitt, R.: 1942. The transmission of Plasmodium lophurae, an avian malaria parasite, by Anopheles quadrimaculatus. Pub. Health Reps. 57:1891

Jacobi, L.: 1939. Beiträge zur Pathologie der Infektion des Huhnes mit Plasmodium gallinaceum in (Brumpt). Arch. f. exper. Path. u. Pharmakol 191:482.

James, S. P.: 1939. The incidence of exo-erythrocytic schizogony in Plasmodium gallinaceum in relation to the mode of infection. Trans. Roy. Soc Trop Med. and Hyg. 32:763.

_____, and Tate, P.: 1937. New knowledge of the life-cycle of malaria parasites. Nature 139:545

_____, and Tate, P.: 1938. Exo-erythrocytic schizogony in Plasmodium gallinaceum Brumpt, 1935. Parasitology 30:128.

Kikuth, W.: 1931. Immunbiologische und chemotherapeutische Studien an verschiedenen Stämmen von Vogelmalaria. Zentralbl. f. Bakt. I. Orig. 121:401.

Kraneveld, F. C., and Mansjoer, M.: 1953. Onderzoekingen over voorkomen van Plasmodium gallinaceum (Brumpt, 1935) in Indonesia. Hemera Zoa 60.234

Krishnamurti, P. V., Peardon, D. L., Todd, A. C., and McGibbon, W. H.: 1962. A Plasmodium from chickens in Wisconsin. Poultry Sci. 41:685.

Laird, R. L.: 1941. Observations on mosquito transmission of Plasmodium lophurae. Am. Jour. Hyg. 34:163, Sec. C.

_____, and Lari, F. A.: 1958. Observations on Plasmodium circumflexum Kikuth and P. vaughani Novy and MacNeal from east Pakistan. Jour. Parasit. 44:136.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn., 412 pp.

_____, and Hanson, H. C.: 1953. Blood parasites of the Canada goose. Jour. Wildlife Mgt. 17:185.

Manresa, M., Jr.: 1953. The occurrence of phanerozoites of Plasmodium lophurae in blood-inoculated turkeys. Jour. Parasit. 39:452.

Manwell, R. D.: 1933. The behavior of the avian malaria parasites in the common fowl, an abnormal host. Am. Jour. Trop. Med. 13:97.

_____: 1935. How many species of avian malaria parasites are there? Am. Jour. Trop. Med 15:265.

_____: 1938. The identification of the avian malarias. Am. Jour. Trop. Med. 18:565.

_____: 1945. Malaria infections in four species of Plasmodium in the duck and chicken, and resulting parasite modifications. Am. Jour. Hyg. 38.211.

_____: 1952. Turkeys and ducks as experimental hosts for Plasmodium hexamerium and P. vaughani. Exper. Parasit. 1:274.

_____, and Goldstein, F.: 1939. Exoerythrocytic stages in the asexual cycle of Plasmodium circumflexum. Am. Jour. Trop. Med. 19.279.

_____, and Herman, C.M.: 1935. The occurrence of the avian malarias in nature. Am. Jour. Trop. Med. 15:661.

Mathey, W. J.: 1955a. Two cases of Plasmodium relictum infection in domestic pigeons in the Sacramento area. Vet. Med. 50:518.

_____: 1955b. Malaria in canaries. Vet. Med. 50.569.

Mielcarek, J. E.: 1954. The occurrence of Plasmodium relictum in the wood duck (Aix sponsa). Jour. Parasit. 40.232.

Pelaez, D, Barrera, A., De La Jara, F., and Perez Reyes, R.: 1951. Estudios sobre hematozoarios II. Interés de las investigaciones sobre el paludismo en los animales. Rev. Palud. y Med. Trop. 5:59.

Perez Reyes, R., and Pelaez, D.: 1953. Estudios sobre hematozoarios IV. Comportamiento de una cepa de Plasmodium relictum en Palomas. Rev. del Inst. Salubr. y Enferm. Trop., (Mexico) 13:111.

Rao, S. B. V., Das, J., and Ramnani, D. R.: 1951. Fowl malaria. Indian Vet. Jour. 28.99.

Roudabush, R. L.: 1942. Parasites of the American coot (Fulica americana) in central Iowa. Iowa St. Coll. Jour. Sci. 16:437.

Sergent, Ed., and Sergent, Et.: 1904. Sur les hématozoaires des oiseaux d'Algérie. Compt. Rend. Soc. Biol, Paris 56:132.

_____, Sergent, Et., and Catanei, A.: 1931. Paludisme des oiseaux. Caractères spécifiques des Plasmodium avaires. Arch. Inst. Past. Alger. 9:399.

Stauber, L. A., and van Dyke, H. B.: 1945. Malarial infections in the duck embryo. Proc. Soc. Exper. Biol. and Med. 58:125.

Terzian, L. A.: 1941a. Studies on Plasmodium lophurae, a malarial parasite in fowls. 1. Biological characteristics. Am. Jour. Hyg. 33:1, Sec. C.

Teznan, L. A.: 1941b. Studies on *Plasmodium lophurae*, a malarial parasite in fowls. II. Pathology and effects of experimental conditions. Am. Jour. Hyg. 33:33, Sec. C.

Trager, W.: 1942. A strain of the mosquito *Aedes aegypti* selected for susceptibility to the avian malarial parasite *Plasmodium lophurae*. Jour. Parasit. 28:457.

Vargas, Luis, and Beltran, E.: 1941. *Culex quinquefasciatus*, a new vector of *Plasmodium gallinaceum*. Science 94:389.

Wiselogle, F. Y.: 1946. A Survey of Antimalarial Drugs. 3 vols. J. W. Edwards, Ann Arbor, Mich.

Wolfson, F.: 1938. The common duck as a convenient experimental host for avian plasmodium. Am. Jour. Hyg. 28:317.

——.: 1941. Avian hosts for malaria research. Quart. Rev. Biol. 16:462.

AEGYPTIANELLA INFECTIONS

This protozoon was seen by Balfour (1907 and 1914) in Sudanese fowls. His first interpretation of the organism was that it represented intracellular developmental phases of the fowl spirochaete (*Treponema anserinum*) which accompanied the infection Hindle (1912) and Wenyon (1926) suggested that the intracellular granular bodies represented portions of nuclei extruded into the cytoplasm of the red cell as a result of concomitant spirochaete infection. Balfour (1914) and Carpano (1929) believed that the spirochaete infection and the infection with the granular bodies in the blood cells were distinct, for either could occur in the absence of the other Carpano, who found the organism in both chickens and geese in Egypt, named the organism *Aegyptianella pullorum*, and considered it a piroplasm (Fig. 37.9).

Carpano's definition of the genus Aegyptianella is as follows: Protozoa of red cells: of small size; in shape, rounded, oval, or pyriform, not producing pigment; producing no change in size or shape of infected cell; multiplying in circulating blood by schizogony, producing up to 25 minute merozoites.

A. pullorum can vary in size from 0.5μ–4.0μ, depending upon the stage of development. The parasites are infrequently found in the circulating blood, and when found are present for only a day or two, but may be encountered in the lungs, heart, liver, spleen, and bone marrow. In life the parasites may show slow movements of translation. Since the organisms stain with difficulty and the size is small, morphological study is difficult.

Pathogenicity. As was previously noted,

A. pullorum is usually found in association with the fowl spirochaete in Africa. Carpano observed pure infection of both organisms, however, and found that subinoculations with the pure protozoan infection at no time produced spirochaetes.

The protozoan infection may appear in the acute, subacute, or chronic form. Native fowls show the chronic form. Imported birds which become infected are ill for a few days and then succumb. Fowls crossed with foreign strains show the subacute or chronic form.

The symptoms are ruffing of the feathers, anorexia, hyperthermia, immobility, drooping, paralysis of the joints, and often diarrhea. Necropsy shows anemia, swollen spleen, enlarged liver, yellowish-green kidneys, punctiform hemorrhage in the serosa, and sometimes an infiltration of gelatinous hemorrhage in the coronary sulcus.

Brumpt (1930) reported that splenectomy of fowls recovered from heavy infections caused a reappearance of the parasites in the blood. The fowls recovered from this artificially induced relapse.

Host-specificity. The infection is found naturally in geese and chickens. Experimental attempts to transmit it to ducks, pigeons, and canaries have been unsuccessful.

Transmission. Carpano believes that the frequent presence of Treponema and Aegyptianella in the same bird indicates that *Argas persicus*, the tick transmitter of *T. anserinum*, is also the vector of *A. pullorum*. According to Reis and Nobrega (1936), Coles and Bedford demonstrated carriage by *A. persicus*, although Chaillot and Saunie in 1932 could not confirm it. Coles (1939a) states that only adults of this species act as transmitters.
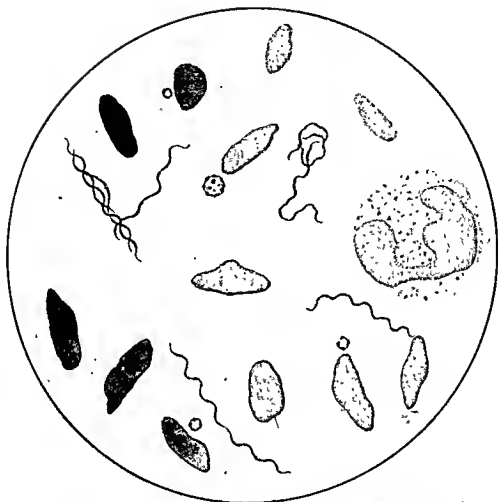
FIG. 37.9 — Aegyptianella pullorum and Spirochaeta gallinarum. (Carpano.)

Geographical distribution. What appears to be this infection has been reported from Egypt, the British and French Sudan, Tunis, South Africa, Transcaucasia, Greece, Yugoslavia, and Albania.

Organisms resembling *A. pullorum* have been described by several investigators under a variety of names. Laird and Lari (1957), who found one of these forms in the heart blood of an Indian house crow, have reviewed the literature on the avian babesiods, compared the findings of the several investigators, and suggest that all of these organisms should be included in the genus Babesia. They liken the organisms found by Schurenkova (1938) and Henry

(1939), Toumanoff (1940), and Mohamed (1952), in the eagle, chicken, heron, and Egyptian kestrel, respectively, to that found in the crow, and suggest that all be considered as belonging to "a single polymorphic species," *Babesia moshkovskii* (Schurenkova, 1938). Levine (1961), for convenience and pending further details on life cycles, prefers to retain *Aegyptianella pullorum* as the name of the organism described above, and considers the rest under the name *A. mushkovskii* (Schurenkova, 1938) Poisson, 1953. In doing so, he includes as organisms of uncertain relationships those of Coles (1937), Abdussalam (1945), McNeil and Hinshaw (1944), and

Rousselot (1917). These latter were found in chickens, turkeys, and pheasants, and could be found to be of veterinary importance, presumably. The accounts of Laird and Lari (1957) and Levine (1961) should be consulted by those interested in studying the avian babesoids in detail.

REFERENCES

Abdussalam, M.: 1945. Piroplasmosis of the domestic fowl in northern India. Indian Jour. Vet. Sci. 15:17.

Balfour, A.: 1907. A peculiar blood condition, probably parasitic, in Sudanese fowls. Jour. Trop. Med. and Hyg. 10:153.

——: 1911. The role of the infective granule in certain protozoal infections as illustrated by the spirochaetosis of Sudanese fowls. Jour. Trop. Med. and Hyg. 14:113.

——: 1914. Notes on the life cycle of the Sudan fowl spirochaete. Trans. Seventeenth Internat. Cong. of Med. (London), Sec. 21, Trop. Med. and Hyg., Part 2, p. 275.

Brumpt, E.: 1930. Réunies parasitaires intenses, dues à la splénectomie, au cours d'infections latentes à Aegyptianella, chez la poule. Compt. Rend. Acad. Sci. 191:1028.

Carpano, M.: 1929. Su di un Piroplasma osservato nei polli in Egitto ("Aegyptianella pullorum") Nota preventiva Clin Vet Milan. 52·339 Also, Bollettino No. 86, Ser. Tec. C Sci., Min. Agr., Cairo, Egypt

Coles, J. D. W A : 1937. A new blood parasite of the fowl. Onderstepoort Jour. Vet. Sci. An. Ind. 9.501.

——: 1939. Aegyptianellosis of poultry. Proc. Seventh World's Poultry Cong, Cleveland, p. 261.

Henry, C. H.: 1939 Presence dans les hématies de poulets d'éléments rappelant les corps de Balfour Bul. Soc. Path. Exot. 32:145.

Hindle, E.: 1912 The inheritance of spirochetal infection in Argas persicus. Proc. Cambr. Philos. Soc. 16:457

Laird, M., and Lari, F. A.: 1957. The avian blood parasite Babesia moshkovskii (Schurenkova, 1938), with a record from Corvus splendens Vieillot in Pakistan. Canad. Jour. Zool. 35:785.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co, Minneapolis, Minn., 412 pp.

McNeil, E., and Hinshaw, W. R.: 1944. A blood parasite of the turkey. Jour. Parasit. 30 (Suppl)9.

Mohamed, A. H. H.: 1952. Protozoal blood parasites of Egyptian fowls. Thesis, London Sch. Hyg. Trop. Med.

Reis, J., and Nobrega, P.: 1956 Tratado de Doenças das Aves. São Paulo, Brazil.

Rousselot, R.: 1917 Parasites de sang de divers animaux de la region de Teheran. Arch. Inst. Hessarek 5:62.

Schurenkova, A.: 1938. Sogdianella moshkovskii gen. nov. sp. nov—A parasite belonging to the Piroplasmidae in a raptorial bird: Cypaêtus barbatus L. Med Parazitol. Parazitar. Bolezni 7 932.

Toumanoff, C 1940 Le parasite sanguin endoglobulaire du héron cendré de l'Indochine (Ardea cinerea var. rectirostris Gould). Babesia (Nicollia) ardeae nov. sp. Rev. méd. franç. d'extrème orient 19.491.

Wenyon, C M 1926 Protozoology. Ballière, Tindall, and Cox, London

SPIROCHETE INFECTIONS

Although spirochetes were discussed in this chapter in earlier editions, they are not protozoa, and are now dealt with in Chapters 14 and 41 of this book.

TRYPANOSOMA INFECTIONS

Trypanosomes have been reported as occurring naturally in a large number of wild birds, and in the chicken, pigeon, and guinea fowl. In most instances the organism was known only from the vertebrate host, and frequently it was designated as a new species if its morphology differed from that of trypanosomes previously reported from birds. Sometimes the organism observed was named as a new species merely because a similar trypanosome had not previously been reported from that particular host. It is now known that most of the trypanosomes of birds can vary considerably, morphologically, and that some, at least, are not specific as to the vertebrate host. They may, indeed, show considerably more specificity for the invertebrate host (see Bennett, 1961). Pending additional studies, it may be well to regard them as Levine (1961) has, who observed, "They all look very much alike and probably belong to a relatively few species. However,

extensive cross transmission studies are needed to establish their relationships and, until these are carried out, it is probably best to refer to them by the names under which they were first described."

Trypanosoma avium Danilewsky, 1885. This trypanosoma was first reported from owls and roller birds in Europe. Since then, trypanosomes regarded as being of this species have been reported by numerous workers. Among the recent reports are those of Diamond and Herman (1954), who found it in Canada geese; Baker (1956a), who obtained it from rooks (*Corvus frugilegus* L.) and jackdaws (*C. monedula spermologus* Vieill.); and Bennett (1961), who observed it in more than 30 species of birds belonging to more than a dozen orders. Diamond and Herman described a technique "for the cultural isolation of trypanosomes from avian bone marrow obtained from living birds or at autopsy." They found the method to be vastly superior to that of merely examining stained smears, or of culturing heart blood, as a means of detecting trypanosome infections. Consequently, reports of the incidence of infection with avian trypanosomes probably have little meaning unless the methods of detection are comparable, or can be brought to comparable bases.

Baker (1956a) gave a good description of the strain of *T. avium* used in his studies, and included references to all pertinent literature. The morphological characteristics of the trypanosome varied somewhat according to the vertebrate host, as well as showing the usual variation within a given host, but in summary Baker describes his flagellate as follows:

"The trypanosome in question is a large spindle-shaped form, measuring an average of 48.2μ in length (excluding flagellum) and 5.5μ in width. There is a tapering flagellar region extending 14.1μ (on the average) beyond the kinetoplast. This trypanosome is assigned to the species *T. avium* Danilewsky, 1885."

Bennett (1961), who also reviewed the literature, including the work of Baker, tabulated measurements for the trypanosomes he found in several species of birds, and came to similar conclusions regarding the morphology and identity of the organism, but recognized that "many physiologically distinct strains or species may exist" in what he termed the *"avium* complex."

The natural methods of transmission of the trypanosomes presently considered to be members of this "complex" are by no means clear. Baker (1956b) described experimental transmission by the hippoboscid *Ornithomyia avicularia* L. He concluded, "The metacyclic trypanosomes develop in the insect's hind-gut, and infect birds by penetrating the membranes of buccal cavity and/or esophagus and crop." Transmission did not occur by the bite of infected louse flies, and he had only very limited success with efforts to produce infections in canaries by rubbing fecal matter from infected flies into scarifications in the skin. Bennett (1961) obtained quite different results using infected mosquitoes (*Aedes aegypti*) and ornithophilic simulids. Infections could be produced by ingestion of macerated insects, but not by feeding the insects intact. Emulsions of material from the hind-gut or feces of infected insects readily produced infections after having been rubbed into scarifications in the skin. The flagellates apparently were unable to penetrate unbroken membranes. However, trypanosomes from some species of birds were quite unable to develop in some insects that successfully transmitted the flagellates acquired from birds of other species. Bennett summed up the situation by commenting as follows: "New criteria for the identification of avian trypanosomes must be developed. It is suggested that the ability of the trypanosome to develop in and produce infective flagellates in a variety of bloodsucking Diptera, whether or not such Diptera are true vectors, be used as criteria. Other criteria, such as serological evidence, or perhaps, the ability to develop on cultures lacking certain chemicals, may yet be needed to definitely separate the species."

Baker (1956c) has given for *Trypanosoma avium* the most complete description of the life cycle in both the vertebrate and

FIG 37.10 — Trypanosoma hannai. (H. de B. Aragão)

invertebrate host that has thus far been presented for an avian trypanosome. Although this species is not known to occur in chickens, it is quite common in Canada geese, and has been transmitted to domestic ducks. In view of the intricate relationships between it (or its "complex") and the many vertebrate and invertebrate hosts from which it has been reported, natural infection of domestic birds can hardly be ruled out. Like infections with other avian trypanosomes, it would probably be of little or no economic importance. *T. avium* has been considered at length here because it is the only avian trypanosome that have received much attention in recent years.

The species mentioned below are included principally because they were described from domestic birds. Little is known concerning their life cycles.

Trypanosoma hannai Pittaluga, 1904. Hanna in 1903 observed a trypanosome in scanty numbers in the blood of domesticated pigeons in India (Fig. 37.10). It was named *Trypanosoma hannai* by Pittaluga (1905). Aragão (1927) also observed the parasite in the blood of a pigeon in Brazil.

The mode of transmission is still unknown, although Aragão noted numerous flagellates of the crithidia type in the alimentary tracts of hippoboscid flies, *Lynchia maura*, which had fed upon a pigeon infected with trypanosomes, but none in flies that had fed upon uninfected birds. Two types of crithidias were noted, the respective dimensions being 40.0μ by 3.0μ and 49.0μ by 1.5μ. Attempts to transmit the infection to clean pigeons by the bite of the fly, by injection of the emulsified intestines of infected flies, or even by blood inoculation were unsuccessful.

Trypanosoma gallinarum Bruce, Hamerton, Bateman, Mackie, and Bruce, 1911. This trypanosome, and another described about the same time by Mathis and Leger (1911) under the name *Trichomonas calmelli*, were both found in chickens. The flagellate described by Bruce et al. (1911) was about $1.5 \times$ as long, and slightly broader than that of Mathis and Leger, but many years ago Wenyon (1926) suggested that they were possibly the same trypanosome. Bennett (1961) went even further, suggesting that both *T. gallinarum* and *T. calmelli* could be considered as belonging to the complex presently regarded as *T. avium*.

REFERENCES

Aragão, H. de B. 1927. Evolution de l Haemoproteus columbae et du Trypanosoma hannai dans la Lynchia maura Bigot. So Biol. C. R. 97:827.

Baker, J. R.: 1956a. Studies on Trypanosoma avium Danilewsky, 1885. I. Incidence in some birds of Hertfordshire. Parasitology 46:308.

——. 1956b. Studies on Trypanosoma avium Danilewsky, 1885. II. Transmission by Ornithomyia avicularia L. Parasitology 46.521.

——. 1956c. Studies on Trypanosoma avium Danilewsky, 1885. III. Life cycle in vertebrate and invertebrate hosts. Parasitology 46:335.

Bennett, G. F.: 1961. On the specificity and transmission of some avian trypanosomes. Canad. Jour. Zool. 39:17.

Bruce, D., Hamerton, A. E., Bateman, H. R., Mackie, F. P., and Lady Bruce: 1911. Trypanosoma gallinarum n. sp. Rep. Sleep. Sickn. Com. Roy. Soc. 11:170.

Diamond, L. S., and Herman, C. M.: 1954. Incidence of trypanosomes in the Canada goose as revealed by bone marrow culture. Jour. Parasit. 40:195.

Hanna, W.: 1903. Trypanosoma in birds in India. Quar. Jour. Micr. Sci. 47.433.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn., 412 pp.

Mathis, C., and Leger, M.: 1911. Recherches de parasitologie et de pathologie humaines et animals au Tonkin. Masson et Cie., Paris. 451 pp.

Pinaluga, D.: 1905. Estudios acerca de los Dipteros y de los parasitos que transmiten al hombre y a los animales domesticos. Rev. Acad. Ciencias, Madrid 3.292, 402, 505.

Wenyon, C. M.: 1926. Protozoology. Ballière, Tindall, and Cox, London.

INTESTINAL FLAGELLATES

Trichomonas Infections

Trichomonads are flagellated protozoa that are characterized by the possession of a longitudinal axial rod, the axostyle, an undulating membrane bordered by a posteriorly directed flagellum, and 3 to 5 "free" flagella that arise (as does the marginal flagellum) from basal granules near the front end of the body. Some students of this group of protozoa have attempted to classify the trichomonads on the basis of the number of "free" flagella characteristic of the genus, while others have regarded the nature of the axostyle, the undulating membrane, and other organelles as better criteria for grouping the trichomonads. For our purposes we may consider all species that are likely to be found in domestic poultry as of the genus *Trichomonas* except for one cecal form for which the name *Tritrichomonas eberthi* has been generally accepted. The reader who has a special interest in presumed evolutionary relationships and the systematics of the trichomonads and related protozoa should consult Honigberg (1963) and some of the papers mentioned in his extensive list of references.

The principal structural components of Trichomonas are designated in Fig. 37.12, which depicts *T. gallinae* semidiagrammatically. Figure 37.11 shows, diagrammatically, how two other trichomonads of poultry differ in details, from each other, and from *T. gallinae*. These are *Trichomonas gallinarum* and *Tritrichomonas eberthi*, mentioned above.

Trichomonas gallinarum Martin and Robertson, 1911. This trichomonad was said by Martin and Robertson (1911) to be one of the most common of the flagellates of fowls. McDowell (1953) found it in more than 60 per cent of "several hundred fowl of varying ages and from many sources." Chickens and turkeys are the hosts from which *T. gallinarum* is most frequently reported, but it has been found in the guinea fowl by several observers, and in chukar partridges by Wichmann and Bankowski (1956). Diamond (1957) found "a *T. gallinarum*-like form" in the Canada goose, and the senior author has observed trichomonads resembling this species in the ceca of pheasants raised in captivity.

T. gallinarum varies considerably in both shape and size. Usually it is pyriform, but sometimes many or most of the individuals may be spherical or slightly elongate. Living organisms viewed in wet smears or hanging drops commonly range in length 7μ–15μ and in breadth 4μ–10μ. The axostyle extends well beyond the body and is slender and pointed. The undulating membrane is prominent, traversing the entire length of the body and ending near the point at which the axostyle emerges. The marginal flagellum often extends well beyond the axostyle. Characteristically, there are 4 anterior flagella which, in living specimens, are best seen by the use of a dark-field or phase-contrast microscope. For a detailed description of structures visible in stained preparations, see McDowell (1953).

Although the usual habitat of *T. gallinarum* is the cecum, organisms may often be found in the adjacent small intestine. Several observers have reported finding this trichomonad in the liver of chickens, and Wichmann and Bankowski (1956) found it in the liver of chukar partridges that were dead upon arrival at the laboratory. Indeed, most if not all reports of the finding
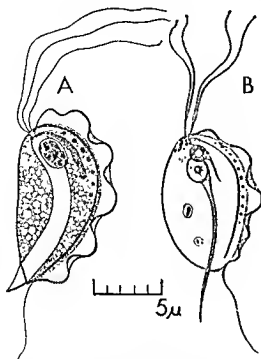
FIG. 37.11 — Diagrammatic representations of the two common trichomonads of the lower digestive tract of domestic birds, as favorable specimens fixed in Schaudinn's fluid and stained with Heidenhain's haematoxylin. A. Tritrichomonas eberthi, B. Trichomonas gallinarum. (Lund.)

FIG. 37.12 — Trichomonas gallinae, semidiagrammatic. a-axostyle. af-anterior flagellum. b-blepharoblast. c-costa. g-cytoplasmic granules. m-mouth. mf-marginal filament. n-nucleus. pb-parabasal body. pf-parabasal fibril. um-undulating membrane. (Stabler, Jour. of Morph., of the Wistar Institute of Anatomy and Biology.)

of *T. gallinarum* in the livers of gallinaceous birds have been based on studies of birds dead long enough to permit postmortem invasion or on studies of birds heavily parasitized by *Histomonas meleagridis*. The livers of birds so parasitized were probably only secondarily invaded by the trichomonad. Rarely, trichomonads may escape into the coelomic cavity and multiply profusely on the serous membranes. Such was the origin of strain TG79 (Soc. Protozool. Comm. on Cultures, 1958). In that instance, apparently, ulceration of the cecum attributable to histomoniasis provided the trichomonads access to the peritoneal and pleural cavities (senior author's unpublished observations and records). It is questionable that *T. gallinarum*, unaided, may invade sites other than the ceca and

adjacent intestine. McDowell's guarded statement (1953) concerning the possible ill effects of heavy cecal infections may approximate the impressions of many workers who have studied farm flocks of either chickens or turkeys. He comments as follows: "The present study discloses that there is a definite association of a yellowish foamy discharge from the caeca and the presence of many *T. gallinarum* in both young fowl and adults, but this diarrhea is most common on range in June and July when every two- to three-month-old fowl will show bedraggled feathers and general unthriftiness as indications of this disease. Severe emaciation may ensue followed occasionally by death." There does, indeed, appear to be a "definite association," but even if a specific parasitical disorder such as hexamitiasis is ruled out, the presence of harmful bacteria or viruses must be considered.

Inasmuch as trichomonads do not form cysts, infection by the ingestion of trophozoites in contaminated food or water must be assumed. However, the intervention of insects, such as flies, as mechanical carriers has long been postulated.

The vast body of literature on the physiology of the trichomonads has been reviewed by Shorb (1964).

Tritrichomonas eberthi (Martin and Robertson, 1911) Kofoid, 1920. This trichomonad inhabits the cecum of chickens and turkeys, and has once been reported from ducks (Kotlán, 1923). According to McDowell (1953), who found it in 35 per cent of the several hundred chickens that he examined, it occurs principally in older birds, and usually in association with *T. gallinarum* and *Chilomastix*.

The body of *T. eberthi* was described by McDowell as "carrot-shaped," with an average length, in fixed specimens, of 13μ, and a width of 6.5μ. There is considerable variation in size, however, and spherical forms sometimes occur. The undulating membrane is prominent, and traverses virtually the entire length of the body. The marginal flagellum trails well behind. In these respects *T. eberthi* resembles *T. gallinarum*. However, it is frequently rather larger, and the cytoplasm is more heavily vacuolated than that of *T. gallinarum*. Also, the axostyle of *T. eberthi* is more massive and tends to taper more abruptly at its posterior end than that of *T. gallinarum*. Usually, only 3 anterior flagella can be demonstrated, and according to McDowell, it is their beating in unison that imparts to the organism its jerky movement. McDowell also gives in considerable detail the distinctive internal features visible in stained preparations.

T. eberthi is nonpathogenic, and the modes of transmission are assumed to be similar to those for *T. gallinarum*. Diamond (1957) cultivated *T. eberthi* axenically for the first time.

Trichomonas gallinae (Rivolta, 1878) Stabler, 1938. Synonyms, *T. columbae* auct.; *T. hepaticum* (Rivolta, 1878); *T. diversa* Volkmar, 1930; *T. halli* Yakimoff,

1934. According to Stabler (1938a), the turkey and pigeon trichomonad of the upper digestive tract are identical species, and (Stabler, 1938b) the correct name is *T. gallinae* (Fig. 37.12) The flagellate occurs almost universally in pigeons, and this bird is probably the primary host (Stabler, 1951a).

Its occurrence in these birds in America was first reported by Waller (1934), who compiled a brief historical account. Its natural host range includes several varieties of domestic pigeons, the band-tailed pigeon, eastern mourning dove, western mourning dove, ring-necked dove, various hawks, falcons and owls, turkeys and chickens (Stabler, 1941b; Stabler and Herman, 1951; Locke and James, 1962). It can easily be transferred from one of these hosts to another, as from doves to clean pigeons (Stabler, 1951b), or from pigeons to hawks or falcons (Stabler and Shelanski, 1936). Levine et al. (1941) transmitted *T. gallinae* from chickens to the turkey, quail, canary, and English sparrow with lesions, and to a duckling without lesions. Additional accounts of transfer experiments with *T. gallinae* are given in Stabler's excellent 1954 review of virtually all of the important literature on this organism.

Among the better accounts of the morphology of *T. gallinae* are those of Stabler (1941a, 1954) and Levine and Brandly (1939). The organism is roughly pear-shaped, but may be rounded under adverse conditions. It ranges in length 6μ–19μ, and in width 2.5–9μ, averaging 10.5μ by 5.2μ. As in *T. gallinarum*, there are 4 free anterior flagella, but the body is more elongate, the undulating membrane and costa are shorter, and the marginal flagellum terminates at the end of the undulating membrane as shown in the figure.

T. gallinae of pigeons occurs in the mouth, pharynx, esophagus, and crop of apparently healthy carriers. The squabs become infected through the ingestion of their natural food, "pigeon milk." The infection can probably be spread also through contamination of feed ...ter. The incidence in pigeons and ...

be very high. Stabler (1951a) in Colorado found infection in 19.3 per cent of trapped band-tailed pigeons, 23 per cent of western mourning doves, and 69 per cent of captured common pigeons. The diseased conditions produced in the hosts, as described by Stabler (1947) and Stabler and Herman (1951), vary considerably. In chronic infections there are no lesions, but in mild cases the apparently healthy bird may show "small, yellowish, adherent masses in the oral or upper oesophageal regions of the digestive tract," which may disappear later. More severe cases frequently end in death, even certain cases where the lesions appear to be slight and confined to the mouth and pharynx. First, yellowish lesions appear on the oral mucosa. These grow into large caseous lumps which may prevent swallowing food or drink. The bird wastes away and may die on about the eighth day of the infection. Pigeon fanciers call the disease "canker," and Stabler states that falconers refer to the condition as "frounce." Involvement of the internal organs may be noted at necropsy, particularly of the liver, which may show only a few yellowish necrotic spots, or more extensive caseation. Heavy losses from the disease have been reported in wild pigeons and pigeon colonies.

Wild mourning doves are frequently victims of this form of trichomoniasis. The 1950 Alabama outbreak killed many thousands of these birds (Haugen, 1952). Most of the deaths occurred after the spring migration or during the nesting season. The dead birds were emaciated and showed throat swellings that probably made it impossible for the stricken birds to swallow food or drink. Herman (1953) has prepared a brief paper on recognition of the disease in doves.

T. gallinae infections in turkeys on range frequented by pigeons are fairly common. In some flocks losses may be considerable. A discussion of trichomoniasis of the upper digestive tract of turkeys appears in Chapter 41, Diseases of the Turkey, in this volume. Levine and Brandly (1939, 1940)

have reported on the disease in pullets and chicks.

Strains of the organism of varying degrees of virulence have been reported (Stabler, 1948a). Of five strains, each passed serially through five Trichomonas-free pigeons, one produced not the slightest evidence of disease in any of the five recipients; one produced but mild cankers in the throat of three, one killed one bird and produced moderate to severe caseation in the throats of the other four, one killed four and produced moderate caseation in the survivor, while the last killed three and produced very severe caseation in the two survivors. The first four strains stemmed from pigeon infections, the last from a cankerous Peregrine falcon which died of the disease. Stabler (1948b) then proceeded to demonstrate that eight squabs given a very mild strain developed either no or evanescent lesions, and, when challenged with a virulent, killing strain, survived with little or no evidence of pathological changes in seven of them. Later Stabler (1951b) isolated from mourning doves one strain that was avirulent in pigeons, and four others of varying degrees of virulence. When the survivors were challenged with virulent, killing strains, mostly of pigeon origin, not a bird died.

That the virulence of a strain ("Jones' Barn") is retained for a long time is supported by Stabler (1953b), who passed the flagellate in succession through 119 Trichomonas-free pigeons of which 114 died. This strain is so pathogenic that of ten clean pigeons, each administered a single trichomonad by Stabler and Kihara (1954), five became infected and died of canker in 8 to 13 days (av. 11.8 days). The five uninfected pigeons were later given 5 to 10 thousand trichomonads each, and they died within (av.) 7.6 days. Later, 293 deaths were reported in 300 birds (Stabler, 1957).

With flagellates inhabiting any part of the digestive tract, the question always arises: Is the flagellate itself pathogenic, or

is it the accompanying bacteria that are pathogenic? The lesions of *T. gallinae* in the upper digestive tract of pigeons are, of course, accompanied by bacteria, but the caseations of the liver and other internal organs may not be. Bos (1933), Cauthen (1936), and Stabler and Engley (1946) first succeeded in obtaining bacteria-free cultures stemming from such bacteria-free lesions. The last-named authors demonstrated that the microorganism fully retained its pathogenicity when grown in bacteria-free culture on modified Boeck-Drbohlav (1925) medium originally used for cultivating *Entamoeba histolytica*.

Copper sulfate in the drinking water was one of the older and partially successful treatments (Jaquette, 1948). Enheptin in the daily dose of 5.0 mg., administered in a gelatin capsule, was effective in pigeons weighing 350 to 550 gm., even against highly pathogenic strains (Stabler, 1953a). A more convenient method of treatment using soluble enheptin was later described by Stabler *et al.* (1958). This consisted of the use of 6.3 grams of soluble enheptin per gallon of drinking water, continued for 7 to 14 days. Its use was recommended only for nonbreeding birds. The method cannot be used for free-flying flocks that have access to unmedicated water because the medicated water is somewhat unpalatable. On controlled tests, all birds infected with the virulent Jones' Barn strain of trichomonad three days before treatment was started survived, but about 13 per cent still harbored some trichomonads. These were assumed to be the birds lowest in the social hierarchy ("peck-order"), that were permitted only limited access to the medicated water.

Still more recently a drug showing very considerable promise for the treatment of trichomoniasis in several hosts has been used successfully for the treatment of *T. gallinae* infections in pigeons. The drug is metronidazole (1-beta-hydroxyethyl – 2 – methyl–5-nitro–imidazole). According to Bussiéras *et al.* (1961), it prevented mortal-

ity when given orally at the level of 60 mg/kg body weight on 5 consecutive days. Fertility of the breeders was not affected.

Trichomonas anatis (Kotlán, 1923). Synonym, *Tetratrichomonas anatis*, Kotlán, 1923. Kotlán (1923) made a study of the intestinal flagellates of ducks. His work, except for some random observations, is the only information on this subject available. *T. anatis* has a broadly beet-shaped body which measures 13μ–27μ in length and 8μ–18μ in breadth. There are 4 free anterior flagella of about the same length as the body. The well-developed undulating membrane has a sturdy marginal flagellum along its border.

The habitat is the posterior region of the intestine of *Anas boschas dom.* Kotlán states that massive infections are established only when the mucosa is in a catarrhal condition.

Trichomonas anseri Hegner, 1929. Hegner (1929) inoculated three-day-old chicks with cecal material from a goose containing a very few trichomonads. Some of the chicks became heavily infected. The description of the species is based upon the forms which appeared in the chicks. Hegner finds that *T. anseri* has peculiarities which distinguish it from other trichomonads. The body is oval in shape; size, 6μ–9μ by 3.5μ–6.5μ with a mean of 7.9μ by 4.7μ. There are 4 free anterior flagella which arise from 2 blepharoplasts, and a fifth one which forms the border of the undulating membrane and becomes a free lash near the posterior end. The chromatic basal rod is distinct; the axostyle is broad and hyaline and protrudes considerably; the nucleus contains an eccentric karyosome and is otherwise filled with minute chromatin granules. Bacteria are ingested into the cytoplasm through a prominent cytostome.

Chilomastix gallinarum Martin and Robertson, 1911. This flagellate and its cysts were first reported from fowls by Martin and Robertson (1911). The best account is that of Boeck and Tanabe (1926), who also described the process of binary fission in
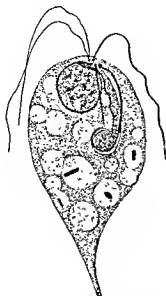
FIG. 37.13 – *Chilomastix gallinarum*, semidiagrammatic, illustrating details of morphology. ×5,000. (Boeck and Tanabe, Am. Jour. Hyg.)

detail (Fig. 37.13) (cf. McDowell, 1953).

The trophozoites of *Chilomastix* are pyriform, somewhat asymmetrical, and plastic. They vary considerably in size, the length ranging commonly 11μ–18μ and the width 5μ–12μ. Occasionally a considerable number of the individuals reach a length of 20μ, or slightly more. After fixation with Schaudinn's fluid the dimensions are 10–20 per cent less. A large cytostomal groove originates near the anterior end and terminates as a pouch just short of the center of the body. It contributes to the asymmetry of the trophozoite, and because it spirals slightly, the organism sometimes seems to be somewhat twisted. The margin of the cytostome appears to be supported by fibrils, but their exact nature, number, extent, and function have been interpreted in various ways. As shown in Fig. 37.13, *Chilomastix* has 3 free anterior flagella, and a fourth flagellum that undulates in the cytostome and aids in the ingestion of bacteria and other food particles. The blepharoplasts which give rise to the flagella, supporting fibrils, and peristomal flagella are difficult to count, but there seem to be 4 of them with the relationships

to the other organelles shown in the figure. The nucleus is rounded and of the vesicular type.

Cysts are infrequently observed in fresh cecal material, but McDowell (1953) asserts that they are common in cultures. They measure 7μ–8.5μ in length and 4.5μ–5.5μ in width. The internal structures are similar to those in the motile form except that the nucleus has moved to a more central position.

For additional details concerning the structure of either the trophozoites or the cysts, as well as for information on a simple method of culturing the organism, the reader is referred to McDowell (1953).

Occurrence and host specificity. *Chilomastix gallinarum* is a common and usually harmless inhabitant of the ceca of chickens and turkeys. McDowell reported having found it in 40 per cent of the "several hundred fowl of varying ages" used in his studies. The senior author's records show that there is considerable variation in the incidence in both chickens and turkeys, from season to season and year to year. The factors responsible for these variations have not been determined. Only rarely is *Chilomastix* present alone, so some of the circumstances favoring infections with trichomonads, amoebae, and coccidia are probably operative.

Chilomastix has also been observed by the senior author in pheasants raised in close confinement, and in one instance in pen-raised chukar partridges. Kimura (1934) reported having found it in ducks, and May (1963) described an outbreak in quail.

Protrichomonas anatis Kotlán, 1923. This species, observed by Kotlán in the duck, has a more or less pear-shaped body measuring 10μ–13μ by 4μ–6μ. There are 3 active flagella which arise from an anterior blepharoplast. It appears as if the axostyle is formed of 2 fibrils which arise from the blepharoplast, pass posteriad, and meet at a point a considerable distance from the caudal tip of the body. The nucleus appears to lie between the fibrils at about the middle of the body.

Cochlosoma anatis Kotlán, 1923. This curious flagellate has a sharply outlined depression at the anterior end of one side. This concavity, similar to the sucking disc of Giardia, marks the ventral surface. The dorsal surface shows a convexity at the anterior end. The body measures $10\mu–12\mu$ by $6\mu–7\mu$. From a blepharoplast on the anterior border of the body there arises a tuft of about 6 flagella, all of which are directed posteriad adjacent to the surface of the body. Two axial fibrils (axostyle?) arise from a granule near the blepharoplast and traverse the body to beyond the caudal tip. A vesicular nucleus lies in about the center of the body. Ovoid cysts with 4 or more nuclei are formed. Reproduction is either by binary fission of the trophozoite or by multiplication within the cysts (Fig. 37.14).

This organism was found in the feces and intestinal mucus of a duckling which was suffering with coccidiosis of the intestine, and in the ceca of a growing duck. Travis located them also in the cloaca and large intestine of ducks. Kotlán found the same flagellate in *Nyroca ferruginea* and *Fulica atra*. Pathogenicity of the flagellate has not been proved. Travis (1938) observed it in the wild mallard, shoveller, pintail, lesser scaup, and domesticated mallard. Other species were observed in the magpie and Eastern robin.

Kimura in 1934 found a *Cochlosoma* in Muscovy and white Pekin ducks in California which he named *C. rostratum*. It measured $6\mu–10\mu$ by $3.9\mu–6.7\mu$. McNeil and Hinshaw (1942) have found it throughout the intestinal tract of turkey poults, and in the region of the cecal tonsil in adults. It occurred in turkeys associated with Hexamita or in combinations of Hexamita and Salmonella. The true significance of this parasite in turkey poults or ducklings has not been determined.

A severe outbreak of cochlosomiasis, attributed to *C. anatis*, in poults of two to ten weeks on a turkey farm in Scotland was recorded by Campbell (1945). The birds were affected with a condition clinically and pathologically indistinguishable from infectious catarrhal enteritis due to Hexamita. The symptoms were as follows: intense thirst, frothy diarrhea, depression, ruffled plumage, drooping head, closed eyes, loss of appetite, weakness, coma, and death. Only 2 or 3 days intervened between the first appearance of symptoms and death. Among the findings at necropsy was the atonic intestine with dilations or bullae filled with yellow fluid swarming with the jerkily swimming flagellates. The flagellate was revealed in every fresh case. Trichomonas and Hexamita were also usually present, but *Cochlosoma* predominated.

HEXAMITA INFECTION

Hexamitiasis is of importance principally as a disease of turkeys, and as such, it is adequately discussed in Chapter 41, Diseases of the Turkey. The organism re-
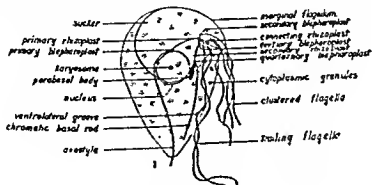
FIG. 37.14 — *Cochlosoma*. Diagrammatic drawing naming structures. (Travis, Jour. of Parasit.)

sponsible for the disease in turkeys, *Hexamita meleagridis* McNeil, Hinshaw, and Kofoid, 1941, has been found in quail, pheasants, and chukar partridges, also (Hinshaw and McNeil, 1941; McNeil, 1958). It can be transmitted experimentally to chickens and ducks, but is not known to occur naturally in these birds. Another species, *H. columbae*, had been described from pigeons (Noller and Buttgereit, 1923) before the specific identity of *H. meleagridis* was set forth by McNeil *et al.* in 1941. *H. columbae* was recognized in California by McNeil and Hinshaw (1941), and Levi (1957) mentions other reports of it in pigeons in California in 1954 and 1956. He calls it "an uncommon protozoan infection" in pigeons, but describes symptons similar to those observed in turkeys. However, in comparison to hexamitiasis of turkeys, that of pigeons is of little economic importance.

The U.S Department of Agriculture (1954) estimated the annual losses attributable to hexamitiasis of turkeys to have averaged about $667,000 for the period 1942–1951 During the decade following that period, the number of turkeys raised in the U.S. doubled (U.S.D.A. Agricultural Statistics 1962), but no indications have been found that losses attributable to hexamitiasis have increased proportionately However, the disease has been reported from areas in which it had previously not been sought or recognized (as in Illinois: Levine, 1961). As McNeil (1958) pointed out, the symptoms of hexamitiasis and bluecomb are very similar, and there may have been some confusion of the two diseases. Furthermore, as she observed, they undoubtedly occur together at times, and it would be of interest to know whether the relationship is more than one of mere coincidence.

A comprehensive paper by Wilson and Slavin (1955) on symptomatology, pathology, and diagnosis of hexamitiasis of turkeys in Great Britain, and a previous paper by Slavin and Wilson (1953), describe a complicated life cycle for *Hexamita meleagridis* involving schizogony, cysts, etc. According to Hoare (1955), this unusual life cycle cannot be accepted without more plausible evidence.

Bacteria-free cultures of *H. meleagridis* in the allantoic cavity of embryonating chicken eggs were obtained by Hughes and Zander (1954), who used rapid passage initially to adapt the flagellates to the new environment, and then the antibiotics streptomycin and bacitracin to eliminate contaminating bacteria. Eight- to 12-day-old embryos proved most satisfactory. No deleterious effects were noted in either the embryos or the chicks which were permitted to hatch.

REFERENCES

Boeck, W. C., and Drbohlav, J: 1925 The cultivation of *Endamoeba histolytica*. Am. Jour. Hyg. 5.371.

——— and Tanabe, M.: 1926. *Chilomastix gallinarum*, morphology, division, and cultivation. Am. Jour. Hyg. 6:319

Bos, A.: 1933. Ueber Trichomoniasis bei Tauben. III Mitteilung: Weitere Beobachtungen uber die Kultur and Pathogenität von Trichomonas columbae. Zentralbl. f. Bakt., I Abt., Orig. 130:220.

Bussières, J, Dams, R., and Euzéby, J: 1961. Prophylaxie de la trichomonose du pigeon par le metronidazole. Bul. Soc. Sci. vét. Lyon 63:307.

Campbell, J. G: 1945 An infectious enteritis of young turkeys associated with *Cochlosoma* sp Vet. Jour. 101:255.

Cauthen, G. E.: 1936. Studies on *Trichomonas columbae*, a flagellate parasitic in pigeons and doves. Am. Jour. Hyg 23 (Jan.).182.

Diamond, L. S.: 1957. The establishment of various trichomonads of animals and man in axenic cultures. Jour. Parasit 43:488.

——— and Herman, C. M.: 1952 *In vitro* studies on avian trichomonads. Jour. Parasit. 38 (No. 4, Sec. 2):11.

Harwood, P. D.: 1946. A fatal case of *Trichomonas gallinae* infection in a nestling mourning dove. Proc. Helminth. Soc. Wash. 13:57.

Haugen, A. O.: 1952. Trichomoniasis in Alabama mourning doves. Jour. Wildlife Mgt. 16:164.

Hawn, M. C.: 1937. Trichomoniasis of turkeys. Jour. Infect. Dis. 61:184.

Hegner, R. W.: 1929. Transmission of intestinal protozoa from man and other animals to parasite-free fowls. Am. Jour. Hyg. 9:529.

Herman, C. M.: 1953. Recognition of trichomoniasis in doves. Bird-Banding. 24:11.

Hinshaw, W. R., and McNeil, E.: 1939. Infectious catarrhal enteritis of turkeys. Turkey Talk 1:5, 7, 21.

————, and McNeil, E.: 1941. Carriers of *Hexamita meleagridis*. Am Jour Vet Res 2.453

Hoare, C. A.: 1955. Life cycle of *Hexamita meleagridis*. Vet Record 67:324

Honigberg, B. M.: 1963. Evolutionary and systematic relationships in the flagellate order Trichomonadida Kirby. Jour. Protozool. 10:20.

Hughes, W. F., and Zander, D. V.: 1954. Isolation and culture of *Hexamita* free of bacteria Poultry Sci. 33:810.

Jaquette, D. S.: 1948. Copper sulfate as a treatment for subclinical trichomoniasis in pigeons Am. Jour. Vet. Res. 9.206.

Jungherr, E., and Gifford, R.: 1944. Three hitherto unreported turkey diseases in Connecticut— erysipelas—hexamitiasis—mycotic encephalomalacia. Cornell Vet. 34 214.

Kimura, G. G.: 1934. *Cochlosoma rostratum* sp. nov., an intestinal flagellate of domesticated ducks. Tr. Am. Micr. Soc. 53:102.

Kotlán, A.: 1923. Zur Kenntnis der Darmflagellaten aus der Hausente und anderen Wasservögeln. Zentralbl. f. Bakt. I. Orig. 90:24.

Levi, W. M.: 1957. The Pigeon, Levi Publ. Co., Sumter, S C.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn., 412 pp.

————, Boley, L. E., and Hester, H. R.: 1941. Experimental transmission of *Trichomonas gallinae* from the chicken to other birds. Am. Jour. Hyg. 33.23.

————, and Brandly, C. A.: 1939. A pathogenic Trichomonas from the upper digestive tract of chickens. Jour. Am. Vet. Med. Assn. 95:77.

————, and Brandly, C. A.: 1940. Further studies on the pathogenicity of *Trichomonas gallinae* for baby chicks. Poultry Sci. 19-205.

Locke, L. N., and James, P.: 1962. Trichomonad canker in the Inca dove, *Scardafella inca* (Lesson). Jour. Parasit. 48:497.

McDowell, S., Jr.: 1953. A morphological and taxonomic study of the cecal protozoa of the common fowl, *Gallus gallus* L. Jour. Morphology 92:337.

McNeil, E.: 1958. Hexamitiasis. Merck Agr. Memo. 3 5.

————, and Hinshaw, W. R.: 1941. The occurrence of Hexamita (*Octomitus columbae*) in pigeons in California. Jour. Parasit. 27:185.

————, and Hinshaw, W. R.: 1942. *Cochlosoma rostratum* from the turkey. Jour. Parasit. 28:349.

————, Hinshaw, W. R., and Kofoid, C. A.: 1941. Hexamita meleagridis sp. nov. from the turkey. Am. Jour. Hyg. 34.71, Sec. C.

Marton, C. H., and Robertson, M.: 1911. Further observations on the cecal parasites of fowls, with some reference to the rectal fauna of other vertebrates. Part I. Quart. Jour. Micr. Sci. 57:53.

May, W. O.: 1963. Chilomastix infection in quail. Southeastern Vet. 14:100.

Nöller, W., and Buttgereit, F.: 1923. Über ein neues parasitisches Protozoon der Haustaube. Zentralbl. f. Bakt. I. Ref. 75:239.

Shorb, M. S.: 1964. The physiology of trichomonads. Biochemistry and Physiology of Protozoa. Vol. III. Acad. Press, New York.

Slavin, D., and Wilson, J. E.: 1953. "*Hexamita meleagridis*." Nature 172.1179.

Slavin, D., and Wilson, J. E.: 1958. A catalogue of laboratory strains of free-living and parasitic protozoa. Jour. Protozool. 5:1.

Stabler, R. M.: 1938a. The similarity between the flagellate of turkey trichomoniasis and *T. columbae* in the pigeon. Jour. Am. Vet. Med. Assn. 93 33.

————: 1938b. *Trichomonas gallinae* (Rivolta, 1878), the correct name for the flagellate in the mouth, crop, and liver of the pigeon. Jour. Parasit. 24:553.

————: 1941a. The morphology of *Trichomonas gallinae* (= *columbae*). Jour. Morphology 69:501.

————: 1941b. Further studies on trichomoniasis in birds. Auk 58:558.

————: 1947. *Trichomonas gallinae*, pathogenic trichomonad of birds. Jour. Parasit. 33:207.

————: 1948a. Variations in virulence of strains of *Trichomonas gallinae* in pigeons. Jour Parasit. 34:147.

————: 1948b. Protection in pigeons against virulent *Trichomonas gallinae* acquired by infection with milder strains. Jour. Parasit. 34:150.

————: 1951a. A survey of Colorado band-tailed pigeons, mourning doves and wild common pigeons for *Trichomonas gallinae*. Jour. Parasit. 37:471.

————: 1951b. Effect of *Trichomonas gallinae* from diseased mourning doves on clean domestic pigeons. Jour. Parasit. 37:473.

————: 1953a. Effect of 2-amino-5 nitrothiazole (enheptin) and other drugs on *Trichomonas gallinae* infection in the domestic pigeon. Jour. Parasit. 39:637.

————: 1953b. Observations on the passage of virulent *Trichomonas gallinae* through 119 successive domestic pigeons. Jour. Parasit. 39 (No. 4, Sec. 2):12.

Stabler, R. M.: 1954. *Trichomonas gallinae:* a review. Exper. Parasit. 3:368.

———; 1957. Further observations on the passage of virulent *Trichomonas gallinae* through successive non-immune domestic pigeons. Jour. Parasit. 43(No. 5, Sec. 2):40.

———, and Engley, F. B., Jr.: 1946. Studies on *Trichomonas gallinae* infections in pigeon squabs. Jour. Parasit. 32:225.

———, and Herman, C. M.: 1951. Upper digestive tract trichomoniasis in mourning doves and other birds. Trans. No. Am. Wildlife Conf. 16:145.

———, and Kuhara, J. T.: 1954. Infection and death in the pigeon resulting from the oral implantation of single individuals of *Trichomonas gallinae.* Jour. Parasit. 40:706.

———; Schnittner, S. M., and Harmon, W. M.: 1958. Success of soluble 2-amino-5-nitrothiazole in the treatment of trichomoniasis in the domestic pigeon. Poultry Sci. 37:352.

———, and Shelanski, H. A. 1936. *Trichomonas columbae* as a cause of death in the hawk. Jour. Parasit. 22-539.

Tanabe, M.: 1926. Morphological studies on *Trichomonas.* Jour. Parasit. 12:120.

Travis, B. V.: 1938. A synopsis of the flagellate genus *Cochlosoma* Kotlán, with the description of two new species. Jour. Parasit 24-313.

Uribe, C.: 1921. A common infusion flagellate occurring in the caecal contents of the chicken. Jour. Parasit. 8-58.

U S D A 1954. Losses in agriculture U.S.D.A. Agr Res. Serv. ARS-20-1.

U.S.D.A. 1963. Agricultural statistics, 1962.

Walker, R V L.: 1948. Enterohepatitis (blackhead) in turkeys. I. Canad. Jour. Comp. Med. 12:43.

Waller, E. F.: 1934 A preliminary report on trichomoniasis of pigeons. Jour. Am. Vet. Med. Assn. 84:596.

Wichmann, R. W., and Bankowski, R.A.: 1956. A report of *Trichomonas gallinarum* infection in chukar partridges (*Alectoris graeca*). Cornell Vet. 46-367.

Wilson, J. E., and Slavin, D.: 1955. Hexamitiasis of turkeys. Vet. Record 67:236.

PARASITIC AMEBAS

Entamoeba gallinarum Tyzzer, 1920. This ameba was described by Tyzzer (1920) from the cecal excrement of both young turkeys and the common fowl. The trophozoites measure from 9μ–25μ in diameter; average size, 16μ–18μ. They are continuously and actively motile at room temperature. Pseudopod formation is said by Tyzzer to be gradual rather than eruptive in character, but Hegner (1929b) finds that it is almost as explosive as in *E. histolytica.* The ectoplasm is differentiated from the endoplasm. The latter stains intensely and usually contains a variety of inclusions such as cell fragments, flagellates, amebas of the genus *Endolimax*, and other material from the cecal contents. Tyzzer stated that bacteria are not utilized as food, but McDowell (1953) disagrees. The nucleus is spherical, and measures from 3μ–5μ across. A dense layer of chromatin is closely applied to the nuclear membrane. Tyzzer states that the endosome is centrally located, but in his figures he shows it in an eccentric position (cf. McDowell, 1953).

The cysts are eight-nucleate when mature, but immature quadrinucleate forms occur. The cysts are spheroidal and have an average size of 12μ by 15μ.

This ameba is not known to affect the host adversely in life, but within a short time after death the organisms migrate through the tissue and can be found in large numbers throughout the cecal mucosa and submucosa. Under these conditions the parasite may also ingest epithelial cells. Several workers have found this ameba in blackhead lesions in livers of turkeys along with other microorganisms.

If it should eventually be determined that this *Entamoeba* is identical with that found in the red grouse, the correct name would be *E. lagopodis* Fantham, 1910. Although only four-nucleate cysts of the latter species were noted by Fantham, it is not unlikely that the mature cysts possess eight nuclei. What seems to be *E. gallinarum* was reported from guinea fowls by Hegner (1929b).

Endolimax gregariniformis (Tyzzer, 1920). Synonyms, *Pygolimax gregariniformis* Tyzzer, 1920; *Endolimax janisae* Hegner, 1926.

This small ameba, found by Tyzzer (1920) in the ceca of diseased and normal

REFERENCES

Fantham, H. B.: 1924. Some parasitic protozoa found in South Africa: VII. So. African Jour. Sci. 21:435.

Hegner, R. W.: 1926. *Endolimax caviae* n. sp. from the guinea-pig and *Endolimax janisae* n. sp. from the domestic fowl. Jour. Parasit. 12:146.

——: 1929a. Transmission of intestinal protozoa from man and other animals to parasite-free fowls. Am. Jour. Hyg. 9:529.

——: 1929b. The infection of parasite-free chicks with intestinal protozoa from birds and other animals. Am. Jour. Hyg. 10:33.

Levine, N. D.: 1961. Protozoan Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minn., 412 pp.

McDowell, S., Jr.: 1953. A morphological and taxonomic study of the caecal protozoa of the common fowl, *Gallus gallus* L. Jour. Morph. 92:337.

Tyzzer, E. E.: 1920. Amoebae of the caeca of the common fowl and of the turkey. Jour. Med. Res. 41:199.

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38

Poultry Surgery

Avian surgery was given little or no consideration for many years. The low value of the individual fowl in the farm flock made it economically unsound to resort to individual treatment. In recent years, as the result of the growth and development of the poultry industry to its present large-scale production methods, there has been an increased demand for better and more effective veterinary service to reduce poultry losses to a minimum. The comparatively high individual value of show birds and ornamental and game birds, as well as avian pets, has created considerable demand for individual medication and improved surgical methods. Consequently, substantial progress in avian surgery has been made for practical and experimental purposes.

The avian species make excellent surgical subjects because their sensory nervous systems are apparently not as highly developed as those of the majority of our domesticated animals, and consequently, they suffer less from surgical shock. They also respond to both general and local anesthetics if properly administered. Birds also have a very high resistance to pyogenic infection, which simplifies effective surgical technique under field conditions with little danger of postoperative infection.

Avian surgery may be classified in two distinct groups, practical, or applied, and experimental. The first group includes those operations necessary to relieve certain conditions which are quite commonly encountered in poultry practice. The second group has been developed for the purpose of studying various problems in physiologic and pathologic research. Experimental surgery is of little practical value. It will be treated briefly together with references for the benefit of those desiring this information. Burrows (1936) developed a technique for the removal of the gizzard of the domestic fowl. Sloan (1936) successfully removed the yolk from newly hatched chicks. Durant (1926), Schlotthauer *et al.* (1933), and Delaplane

and Stuart (1933) have developed surgical techniques for cecal abligation in an effort to control infectious enterohepatitis in turkeys. Some of the surgical methods developed in poultry research have contributed much toward our present knowledge of avian physiology and pathology.

Anesthesia. Anesthetics are not generally used in poultry practice. They have been used successfully in experimental investigations which involved major operations. The anatomical structure of the avian respiratory system renders the use of inhalation anesthesia rather unsatisfactory. This type of anesthesia cannot be controlled adequately because of the infiltration of inhaled anesthetic into the air sacs which constitute part of the respiratory system in birds. Frequently, excessive amounts of the anesthetic are absorbed, resulting in respiratory failure, cardiac inhibition, and death. Consequently, ether and chloroform have been replaced by some of the more recently developed anesthetics which can be administered intravenously. Chloral hydrate injected intravenously in doses from 0.2 to 0.1 gm. produces a complete anesthesia lasting from 15 minutes to 1 hour. Hole (1933) reported the minimum lethal dose of chloral to be between 0.1 and 0.6 gm. Gandal (1956) administered chloral hydrate in various ways and found it to be toxic and difficult to control. King and Biggs (1957) reported that the prolonged action and toxicity of urethane made it undesirable for surgical anesthesia. The intravenous administration of sodium amytol solution (0.1 gm. per cc.) is quite satisfactory as a general anesthetic according to Fretz (1952). Injections of 0.5 to 1.0 cc. of this solution is adequate for birds ranging from 4 to 8 pounds in weight. Warren and Scott (1935) consider sodium pentobarbital (nembutal) the most satisfactory general anesthetic for poultry practice. Intravenous injections of 0.5 to 0.75 cc. produce effective anesthesia for as long as 2 hours. The intravenous use of nembutal for turkeys in doses of 1.1 cc. per 5 pounds of body weight was reported by Durant and McDougle (1935) as an effective anesthetic. Arañez and Rosario (1960) reported on the use of kemithal sodium as a general anesthetic for chickens. Friedburg (1962) describes successful techniques for anesthesia of parakeets and canaries. For anesthesia of short duration, ether or ethyl chloride, preferably the latter, is administered by intermittent application. For more prolonged operations he suggested pentobarbital sodium or a chloral hydrate-pentobarbital-magnesium sulfate combination (Equi-Thesin, Jensen-Salsbery Laboratories, Kansas City, Missouri). The dose is 0.25 cc. per 100 gm. body weight. The average parakeet weighs about 50 gm. or 1 oz., and would require about 0.08 cc. in the pectoral muscle. A 1.0 cc. glass tuberculin syringe with a 26 gauge, ¼ inch needle is suitable for the administration of the anesthetic. The average canary weighs about 20 gm. but the exact weight should be obtained for calculation of dosage of anesthetic. Pentobarbital sodium may be used if properly diluted. A concentration of 7 mg. per 1 cc. is suitable. For a 30 gram bird 0.2 cc. of this dilution injected into the pectoral muscle is used. After five to ten minutes, suitable anesthesia lasts about ½ hour.

Local anesthetics have a limited use in poultry practice. Schalm (1936) reported the successful use of 2 per cent solution of novocain as a local anesthetic. Butyn in a 2 per cent solution is regarded very satisfactory by Sweebe (1925) for local anesthesia of the eye.

ABDOMINAL SURGERY

Abdominal surgery is limited to a few operations for conditions which are quite common in poultry practice. Caponizing is probably the most extensive operation performed for the production of birds for a specialized market. The premium received for this class of poultry products makes the operation practical and economically profitable. The removal of eggs in the various stages of development from the abdominal cavity of birds is practiced, especially in cases involving birds

which are known to be high producers or those which have high individual values. The removal of excessive quantities of fluids which accumulate in the abdominal cavity is often indicated. Cecal abligation has been given considerable attention in recent years in an attempt to control blackhead in turkeys. At the present time, this operation must be considered only as an experimental procedure. An effective method of sanitation and hygiene instituted in the system of management has proven to be more practical and satisfactory in controlling this disease. Because of the interest shown in this work, a brief review of some of the methods developed will be presented.

Caponizing. The castration or desexing of cockerels is commonly known as caponizing. The optimum stage of development is reached at 8 to 10 weeks of age when the birds reach about 1½ pounds in weight. At this age there are fewer so-called slips, and the mortality can be kept at a minimum. However, Quigley (1961) recommends caponizing earlier; from 5 to 6 weeks of age. The water should be withheld for 12 to 18 hours prior to operation. This permits the intestinal contents to be reduced to a minimum, causing the intestines to settle away from the testicles as well as the upper wall of the abdominal cavity. Consequently, when the intercostal incision is made, there is a clearer field of operation in the abdominal cavity and less danger of intestinal puncture. A suitable table, proper confinement, adequate light, and proper instruments are essential for satisfactory results.

The cockerel is placed on the operating table on its left side. The wings are securely held together above the body by a suitable restraining device and fastened to the upper side of the table. The legs are likewise secured together and fully extended, being fastened to the lower edge of the table. This position permits free access to the field of operation (Fig. 38.1). Some of the soft feathers are plucked from the region of the hips and ribs. The field of operation is cleansed with a piece of

FIG. 38.1 — Field of operation for caponizing.

cotton moistened with clean warm water or weak antiseptic solution. With the fingers of the left hand, locate the area midway between the last two ribs (6th and 7th) where the incision is to be made. Draw the skin back over the hip and with it the sartorius or thigh muscle with the left hand. An incision is made about ¾ inch long through the skin and intercostal structures, midway between the last two ribs slightly below the upper abdominal wall (Fig. 38.2). If the incision is too near the vertebral column or too low, difficulty may be experienced in locating and removing the genital organs. The incision of the vein which runs diagonally across the body from thigh to wing should be avoided. The spreader is placed in the incision, the handle of which should be
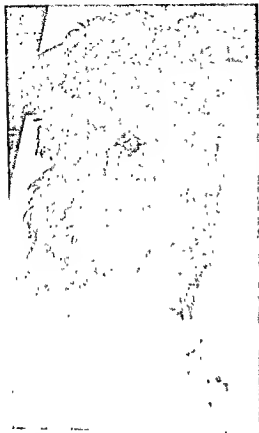
FIG. 38.2 — Incision with insertion of spreaders.

directed toward the back so as not to interfere with the operator. The peritoneal membrane medial to the intercostal structures which forms the abdominal air sac is punctured with a sharp hook or probe exposing the abdominal organs. The gonads, which are about the size of a wheat kernel, can be seen attached dorsally at the anterior extremity of the kidney. These organs are usually yellowish in color but may vary from white to gray or even black. The lower gonad (left) should be removed first by clamping it securely with forceps and twisting it free from its attachments. The upper one (right) is removed in the same way. The spreaders are then removed and the bird is released from the operating table. The skin and thigh muscle slip back in place as the

limbs regain their normal positions, serving effectually to close the opening in the abdominal cavity.

The principal danger in this operative procedure is death from internal hemorrhage which takes place within a few minutes following rupture of a primary blood vessel. The testicles are located at a point where the right and left iliac veins converge into the posterior vena cava (Fig. 38.3). The aorta is situated below the vena cava when the bird is on the operating table. The spermatic vessels which supply the circulation of the testicles are ruptured when the testicles are removed; but in the young cockerel these vessels are so small that serious hemorrhages are seldom experienced. The operative field is such that injury to the major blood vessels can be avoided with ordinary care.

Electrical instruments have been devised and sold on the market for the removal of the gonads to take the place of the more commonly used instruments. They have the advantage of cauterizing the tissue to which the gonads are attached and possibly reduce the number of slips. They are not as easy to manipulate as some other instruments and not suitable for use under field conditions where electricity is not available.

The removal of the entire organ is very essential in order to prevent so-called slips. Any fragments which remain intact may result in the proliferation of this reproductive tissue, and the operation will fail to achieve the desired objectives. The secretions from the incised tissues rapidly form hard crusts or scabs. It takes much longer for the healing of the incised tissue to become complete. A certain percentage of operated birds will develop wind puffs which are caused by the air escaping from the body cavity through the intercostal incision becoming enclosed in the subcutaneous tissues. This condition is relieved by puncturing the skin and releasing the enclosed air. The birds should be transferred to clean houses with plenty of clean litter on the floors. They should be confined and kept as quiet as possible

FIG. 38.3 — Ventral view showing the relative position of the gonads to that of the major blood vessels in the operative field. (1) Left testicle slightly posterior to the right. (2) Vena cava formed by the union of the right and left iliacs (3). (4) Dorsal aorta. (5) Coccygeal vein. (6) Mesenteric artery. (7) Operative incision on the right side through which both the gonads are removed. (Kans. Agr. Exper. Sta.)

for a week or 10 days following caponizing, after which they may be given free range and managed to produce the maximum growth and development.

Considerable work has been done in an effort to produce capons by the use of chemicals rather than by the usual operative procedure. Guinn (1944) reported a method which apparently is quite effective and may prove to be of practical importance. Male birds, ranging in age from 8 to 10 weeks, received a highly compressed pellet containing an average of 15 milligrams of diethylstilbestrol implanted under the skin of the neck. An incision

about one-fourth of an inch long was made in the skin of the neck and the pellet was introduced into the incision and pushed about one inch forward from the point of incision. The treated birds gradually lost all male characteristics and to all external appearances and tissue examination could be considered as true capons. There was no recurrence of male characteristics up to the time at which the birds reached 5 or 6 pounds in weight. Mature male birds receiving diethylstilbestrol pellets showed considerable improvement in body fat and quality of meat. Lorenz (1945) and Watel (1948) found that the

subcutaneous implantation of diethylstil-bestrol was much more effective than the oral administration of this agent. It should be remembered that such subcutaneous implants of any product should be made in the discarded or inedible portions of the bird, such as the upper cervical region. The consumption of the tissue containing either a whole or residual diethylstilbestrol pellet might cause an unfavorable reaction in those who consume such material. Lauffer (1957) found that estrogen treatment was superior to caponizing, producing heavier birds in which the finish, feathering, and fleshing were superior.

The U.S. Food and Drug Administration has not permitted the use of diethylstilbestrol in pellet or paste form in poultry since December, 1959. Residues of this chemical were found in the tissues of treated poultry and such poultry products were considered to be unsuitable for food purposes.

Poulardization. Poulardization is the surgical removal of the ovary in birds. This operation has been performed in poultry for many years but is rarely done. Experimental surgery has shown that the removal of the reproductive capacity of birds hastens the growth and improves the quality of the flesh. Apparently poulardization of ducks has gained limited acceptance in the Philippines recently. The operative technique described by Arañez and Saguin (1955) is quite satisfactory, especially in ducks. Undoubtedly, suitable estrogen treatments could be developed which would achieve the same objectives without surgery or the surgical risks involved.

Cecal abligation. The surgical technique developed for this operation has been reported by Durant (1926) for fowls and at a later date for turkeys with reference to the control of blackhead (Durant, 1930). The general procedure is similar to that for caponizing. The incision is made on the left side between the last two ribs, as the junction of the ceca and the intestine is located opposite this point.

The proximal terminations of the ceca can be easily lifted through the incision to a convenient position for ligation. Each cecum is occluded by means of two catgut ligatures about 4 mm. apart as close as possible to the junction of the ceca and the intestine. These ligatures should be drawn tight enough to close the lumen of the ceca, and yet not tight enough to cut through the cecal wall. The organs are then replaced and the incision is closed by a single suture. Durant reported that if the ligature is passed around the two adjacent ribs and drawn tight enough effectively to close the margins of the incision, the subsequent danger of wind puffs is practically eliminated. The ceca do not become necrotic because the blood supply to these organs is not destroyed. Atrophy of the ceca from disuse follows in most instances, and they become scaled pouches suspended on the mesenteric ligaments. Occasionally, both ceca may become greatly enlarged in from 7 to 32 months after the operation. While the complete occlusion of the ceca was effective in preventing blackhead in turkeys, the mortality was so great as to make the procedure economically unprofitable.

The use of aluminum clamps in place of the cecal ligatures as reported by Delaplane and Stuart (1933) reduced the mortality considerably, but this method did not entirely prevent the ceca from resuming their functional activity.

A different operative technique, which proved to be more successful, was developed by Schlotthauer and his associates. They made the abdominal incision, medial and parallel to the left pubic bone, about 1¼ inches in length. A small aneurysm needle was slipped through the mesentery under the ceca near their proximal terminations and the organs exposed through the abdominal incision. A small hemostat was placed across both ceca through the aperture in the mesentery made by the aneurysm needle, and the cecal walls were completely crushed by the hemostats. Silk ligatures were placed above and below the crushing

clamp. No losses were reported from this operation, and the resumption of functional activity of the ceca was not observed.

Egg retention. The retention of eggs in the posterior portion of the oviduct is not uncommon in fowls during heavy production. This condition may be caused by temporary suspension of the normal physiological activity or may be caused by an obstruction. Abnormally large eggs also may be rather firmly lodged in the oviduct, which require manipulation or surgical removal.

In many cases where there is no indication of pathological conditions, the retained eggs may be removed easily without surgical methods. A lubricant such as mineral oil may be introduced into the oviduct. The forefinger is inserted through the vent; gentle pressure on the abdomen is exerted with the other hand forcing the egg toward the vent. In cases involving eggs of excessive size which cannot be removed without injury to the tissues of the oviduct and cloaca, the egg may be moved posteriorly by manipulation to a position where the shell may be seen through the vent. The shell is then punctured with a sharp instrument, after which the egg contents and shell fragments are removed. The cloaca and posterior portion of the oviduct may be irrigated with a cool, mild antiseptic solution to reduce the inflammatory reaction. In cases where it is apparent that tumors are responsible for the obstruction, the fowl should be destroyed.

Removal of eggs from the abdomen. The accumulation of eggs in the abdominal cavity occurs in fowls during periods of high production. The abdomen becomes greatly distended, often reaching the ground (Fig. 38.4A and B). The feathers are removed from the operative field on the abdomen below the vent. The surface of the skin should be cleansed and disinfected with a mild solution of a suitable antiseptic. General anesthesia may be used effectively. An incision about 3 inches long is made through the skin and abdominal wall between the xiphoid terminal of the sternum and the cloaca. The eggs may be removed from the abdominal cavity by manipulation and pressure on the abdominal wall. The incision is then closed by using a continuous catgut suture. McKenney (1929) reports continuous production following a successful operation. Gandal (1960) describes a practical technique for relief of egg-bound birds. For parakeets, canaries, and smaller birds a serrated-tip thumb forceps is inserted into the cloaca in closed position and then allowed to expand slowly to make
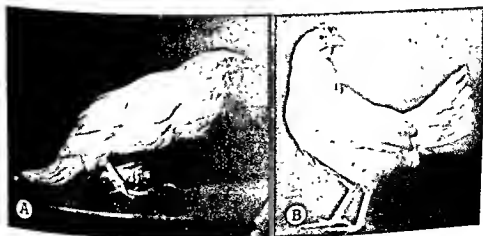
FIG. 38.4 — (A) Characteristic posture of hen with ova in the peritoneal cavity before operation. (B) The hen 8 days after operation. (McKenney, Jour. A.V.M.A.)

applied to the wound. Feed and water should be withheld for about 12 hours, after which the bird should be fed sparingly for a few days.

Crop impaction. The ingestion of large quantities of bulky or dry feed frequently results in the overdistention of the crop and the inhibition of the normal physiological activity. In some cases a rubber tube may be inserted in the esophagus, and the injection of water into the crop may soften the crop contents sufficiently to relieve the condition. When the removal of the crop contents is indicated, the operative technique used in the removal of foreign bodies may be successfully employed. In cases of greatly enlarged or pendulous crops, a portion of the wall may be removed before suturing. The postoperative care of the bird is similar to that described in the discussion of foreign bodies. Fisher and Weiss (1956) found that surgical crop removal in chicks had no untoward effect on subsequent growth and development.

Amputation of comb and wattles. The surgical removal of the comb and wattles is commonly known as "dubbing" and "cropping" in poultry practice. Extensive injury, edema, or infection of these appendages indicates removal. The abnormal development of the combs and wattles frequently makes it advisable to remove them in order to prevent possible injury. The operative technique developed by Schalm (1936) has been highly satisfactory. The combs of birds are very vascular,

and their removal may be followed by profuse and often fatal hemorrhage unless proper operative technique is employed. A suitable local anesthetic such as a 2 per cent solution of novocain in combination with adrenalin or another hemostatic agent can be used effectively. A special clamp for this purpose (Fig. 38.5) is applied to the base of the comb and tightened sufficiently to arrest the circulation of the appendage (Fig. 38.6A). The comb is amputated with a scalpel or scissors one-eighth of an inch above the clamp, following the curvature of the clamp. The cut surface is thoroughly seared with a hot iron and the clamp is removed (Fig. 38.6B). The small posterior portion of the comb remaining is removed and seared (Fig. 38.6B). Complete healing follows as a rule in 30 days.

The wattles may be removed with a heavy pair of shears, followed immediately by the application of a suitable astringent such as iron subsulfate. The arterial hemorrhage can be controlled with hemostatic forceps. These operations can be performed at any age, but less hemorrhage is experienced and more rapid healing is observed in younger birds.

Laurent and Carmon (1959) reported a 2 to 3 per cent increase in egg production and lower feed cost following dubbing White Leghorn pullets.

Trimming of claws and spurs. The trimming of the toenails of poultry and pets, including canaries, parrots, and cage birds, can be done easily with heavy surgical shears. The sharp points and edges

FIG. 38.5 — Dubbing clamp.
(Schalm, Jour. A.V.M.A.)
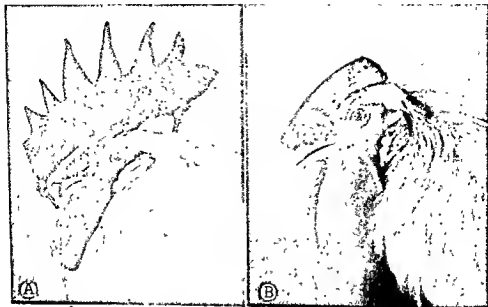
FIG. 38.6 — (A) Cockerel with dubbing clamp applied. (B) Cockerel 24 hours after being dubbed. (Schalm, Jour A.V.M.A.)

should be removed with a flat nail file. The spurs of male birds are frequently removed because of injuries inflicted during fights. During the breeding season injuries to the backs of turkey hens caused by the spurs of the males may be quite serious, and consequently the removal of the spurs is indicated. A pair of canine bone shears is an ideal instrument for this purpose. These appendages can be amputated with little or no hemorrhage if properly done. In turkeys it is well to trim the toenails down almost to the corium. If the toenail should be cut too short, tannic acid powder or any other suitable astringent will effectively control the hemorrhage.

Prevention of spur development. When large numbers of birds in a breeding flock are kept together, it is often advisable to prevent the development of spurs on the male birds. The technique developed by Smith (1932) is simple and effective. This treatment is applied when the spur cap has started to develop and is not over ¼ inch long. Male birds reach this stage of development at 10 to 16 weeks of age, de-

pending on the breed. The spur caps are cut off close to the leg, and a stick of potassium hydroxide is applied and rubbed well into the wound. The cauterizing action of the potassium hydroxide arrests the hemorrhage, destroys the embryonic spur tissue, and prevents the subsequent development of spurs.

Flight control. It is often necessary to arrest the flight of birds either temporarily or permanently. Temporary flight control can be secured by brailing or clipping. Brailing is accomplished by tying one wing closed with a soft cord or bandage. Wing clipping is commonly practiced by poultrymen and consists of cutting the first ten flight feathers close to the wing on one wing only. Clipping is effective in adult birds for a period of about one year while this procedure must be repeated in young birds every month.

The removal of the last segment of the wing at the joint is referred to as pinioning. A tourniquet should be applied to the wing to control hemorrhage. The distal segment of the wing may be removed at the joint with a scalpel or a strong pair

of shears. The wound should be thoroughly cauterized with some good caustic agent to prevent hemorrhage and subsequent infection. This operation should be performed on one wing only and should not be done just prior to or during the breeding season. Sperling and Faber (1951) have recommended wing tip amputation in flight control of game birds.

Permanent flight control may also be obtained effectively by tenectomy, which consists in the removal of a section of the tendon which extends along the under side of the wing and runs parallel to the major blood vessels. Pinioning and tendon resection are recommended for game birds bred in captivity.

Debeaking. The removal of the tip of the upper segment of the beak is known as debeaking. This operation is indicated to control cannibalism and prevent fighting in male birds. The technique developed by Kennard (1937) has been quite effective. The tip of the beak is not cut off but is separated from the deeper structures by traction or tearing. A short cut is made into one side of the beak only, extending into the margin about 1/16 to 1/8 of an inch (depending on the size of the beak) at a point ¼ to ¼ inch posterior to the tip. The flat side of the knife blade is placed against the cut portion of the beak and raised to loosen the edge. The tip is torn off by applying traction toward the opposite side and down toward the lower mandible. This procedure causes little discomfort to the bird and practically no hemorrhage. This removes the tip of the beak, rendering the bird harmless for 2 or 3 weeks. Debeaking does not prevent the bird from eating or drinking nor does it materially affect the egg production in hens. A more recently developed technique, using an improved electrical debeaker, has replaced the surgical removal of the tip of the upper segment of the beak in large-scale poultry production. Debeaking now is a routine process in commercial production, especially the large-scale broiler industry. Lonsdale et al. (1957), Keene et. al. (1959),

and Wyne et al. (1959) found that the removal of one-third of both the maxilla and mandible of day-old baby chicks had no significant effect upon growth and development or feed conversion efficiency. Darrow and Stotts (1954) reported similar experiences. This procedure is effective in the control of various forms of cannibalism.

Draining sinuses of the head. There are several avian diseases associated with infection in the sinuses of the head. In the domesticated fowl the exudate which accumulates in the sinuses rapidly becomes solidified or caseated. In turkeys the exudate remains more or less liquid in form especially in the early stages of sinusitis. The consistency of the exudate can be as certained by palpation of the enlarged sinuses. If the exudate is solidified or caseated, the surgical removal of the exudate and the irrigation of the sinuses are indicated. The incision of the swollen sinus is made at the anterior ventral margin with a pointed scalpel, making an incision from ¼ to ½ inch in length. The exudate may be scraped or curetted out of the sinus and an aqueous solution of silver nitrate applied to the mucous membranes of the sinus. Madsen (1938) reported that a 4 per cent aqueous solution of silver nitrate is slightly more effective than a 2 per cent solution for this purpose. It may be necessary to repeat the application of the silver nitrate solution before the secretion of the exudate is arrested.

The method of treatment recommended by Hinshaw (1937), especially suitable for the treatment of sinusitis in turkeys, consists of the withdrawal of the serous or gelatinous exudate from the sinus with a syringe and hypodermic needle, followed by the injection of silver nitrate solution into the cavities of the sinus. A 5 or 10 cc. syringe fitted with an 18-gauge needle 1½ inches in length is suitable for the aspiration of the exudate from the sinus. The ration of the exudate should be inserted through the skin and sinus membranes into the sinus. The withdrawal of the syringe plunger removes the fluid exudate from the sinus

cavity. About 1 cc. of 4 per cent silver nitrate solution is then injected into the sinus and distributed over the entire surface of the sinus mucosa by gentle massage. Excessive quantities of the silver nitrate solution should be avoided. This method of treatment has been extensively used with satisfactory results. (See sinusitis discussion under turkey diseases.)

Tumors. Neoplastic formations are commonly encountered in poultry practice. According to Feldman (1932), birds are subject to many different types of neoplasms involving practically all tissues and organs. From the clinical standpoint, we are interested in two distinct types—benign and malignant. The benign tumors are localized and develop from a central focus. The malignant tumors spread to the surrounding structures and are capable of establishing multiple foci in the course of their development. Because of the common recurrence of malignant tumors, surgical removal is seldom effective.

Benign tumors may attain such a size as to interfere with the normal physiological processes of the bird. When they occur on the exterior surface or in the superficial structures of the body, they can be removed surgically. Local anesthesia may be employed successfully. The neoplastic tissues are excised and the wounds treated as the local conditions may indicate. Gandal and Saunders (1959) developed a surgical technique for the successful removal of subcutaneous tumors in parakeets. Internal tumor formations are seldom diagnosed in the living bird. In cases where this condition is suspected, it is advisable to destroy the bird.

Fractures. Fractures of the long bones of the wings and legs of birds are not infrequent. Treatment is warranted only in the case of exceptionally valuable birds or in pets. Simple fractures heal readily in a week or 10 days if the fracture is properly reduced and the limb is immobilized. Suitable splints can be fashioned from pieces of light wood, quills, or cork which can be held in place effectively with gauze bandage coated with sodium silicate. In cases of compound fractures, windows may be left in the cast for the purpose of dressing the wound with a powdered antiseptic. Care must be taken not to obstruct the circulation of the limb when applying the splints and bandage.

Blood samples. Blood samples are usually obtained from the wing veins of our domesticated birds. Under certain conditions it is desirable to secure blood samples from various species of the smaller birds. Sooter (1954) used cardiac puncture. McClure and Cedeno (1955) secured blood samples from the right jugular vein. O'Meara (1960) developed a technique by which suitable blood samples could be secured by one person under aseptic conditions. The wings are fastened together above the back with a suitable clamp applied at the primary feather area. The wings are inserted in a narrow slot cut in a cardboard box with the clamp on the underside. The bird's feet may be secured with a cord loop or suitable rubber band placed over the legs. The bird and the support board are placed on a box and the neck of the bird extended over the edge of the support. The suprasternal cleft is cleansed with an alcohol swab, hypodermic needle is inserted and directed from this point posterior and ventral into the heart. The blood sample is slowly aspirated into a small sterile syringe.

REFERENCES

Araßer, J. B., and Rosario, W. B.: 1960. Kemithol sodium as a general anesthetic for chickens. U.P. Vet 4:39.

————, and Saguin, C. S.: 1955. Poulardization of native ducks. Jour. Am. Vet. Med. Assn. 127:314.

Burrows, W. H.: 1936. The surgical removal of the gizzard from the domestic fowl. Poultry Sci. 15:290.

Darrow, M. S., and Stotts, C. E.: 1954. The influence of debeaking broilers upon growth rate, feed utilization and market quality. Poultry Sci. 33 378.

Delaplane, J. B., and Stuart, H. O.: 1933. Cecal abligation of turkers by the use of clamps in preventing enterohepatitis (blackhead) infection. Jour. Am Vet Med. Asn. 83:253.

Durant, A. J.: 1926. Cecal abligation in fowls. Vet. Med. 21:392.

——: 1950. Blackhead in turkeys—surgical control by cecal abligation Mo Agr. Exper. Sta. Res. Bull. 133.

——, and McDougle, H. C.: 1935. Blackhead in turkeys. Mo Agr. Exper. Sta. Bul 315 101

Feldman, W. H.: 1932. Neoplasms of Domesticated Animals. W. B. Saunders Co., Philadelphia

Fisher, H., and Weiss, H. S.: 1956. Feed consumption in relation to dietary bulk and energy level: the effect of surgical removal of the crop. Poultry Sci. 35:418

Fretz, V. C.: 1932. Anesthetizing poultry. Vet. Med. 27:405.

Friedburg, K. M.: 1962. Anesthesia of parakeets and canaries. Jour Am Vet Med Assn 141:1157.

Gandal, C. P.: 1956. Satisfactory general anesthesia in birds. Jour. Am Vet Med Assn 128:557

——: 1960. A practical technic for the relief of eggbound birds. Vet Med. 55:53

——, and Saunders, L. Z.: 1959. The surgery of subcutaneous tumors in parakeets (Melopsittacus undulatus). Jour. Am. Vet. Med. Assn. 134:212.

Guinn, A. H.: 1944. Chemical eapons. Vet. Jour. 100:241.

Hinshaw, W. R.: 1937. Diseases of turkeys. Calif. Agr. Exper. Sta., Bul 613.

Hole, N.: 1933. Chloral hydrate as a general anesthetic for the fowl. Jour Comp Path and Therap. 46:47.

Kaupp, B. F.: 1933. Poultry Diseases. Sixth Ed. Alexander Eger, Chicago. P. 141

Krene, J. H., Tower, B. A., and Watts, A. B.: 1959. A determination of the immediate effects of debeaking day-old broiler type chickens. Poultry Sci. 38:738

Kennard, D. C.: 1937. Chicken vices. Ohio Agr. Exper. Sta., Bimonthly Bul No. 181 22.33

King, A. S., and Biggs, P. M.: 1957. General anaesthesia in the G domesticus for non survival laboratory experiments. Poultry Sci. 36:490.

Lauffer, R. G.: 1957. The effect of caponizing and estrogen treatments on the performance of New Hampshire cockerels. Poultry Sci. 36:376.

Laurent, C. K., and Carmon, J. L.: 1959. The effect of dubbing White Leghorn pullets. Poultry Sci. 38:136.

Lonsdale, M. D., Vondell, R. M., and Ringrose, R. C.: 1957. Debeaking at one day of age and the feeding of pellets to broiler chickens. Poultry Sci. 36 565.

Lorenz, F. W.: 1945. The fattening action of orally administered synthetic estrogens as compared with diethylstilbestrol pellet implants. Poultry Sci. 24:91.

McClure, H. E., and Cedeno, R.: 1955. Techniques for taking blood samples from living birds. Jour. Wildlife Mgt. 19:477.

McKenney, F. D.: 1929. Operative relief for ova in the peritoneal cavity of the chicken. Jour. Am. Vet. Med. Assn. 74:1067.

Madsen, D. E.: 1938. Sinusitis of turkeys. Utah Agr. Exper. Sta., Bul. 280.

O'Meara, D. C.: 1960. A cardiac bleeding technique for small birds. Avian Dis. 4.250.

Quigley, G. D.: 1961. Caponizing chickens. U.S.D.A. Leaflet No. 490. P. 5.

Schalm, O. W.: 1936. Special technics for dubbing and cropping chickens. Jour. Am. Vet. Med. Assn. 89:713.

Schlotthauer, C. F., Essex, H. E., and Mann, F. C.: 1933. Cecal occlusion in the prevention of blackhead (enterohepatitis) in turkeys. Jour. Am. Vet. Med. Assn. 83:218.

Sloan, H. J.: 1936. The operative removal of the yolks from newly-hatched chicks. Poultry Sci 15:25.

Smith, L. W.: 1932. Preventing spur development on male birds. Poultry Sci. 11:241.

Sooter, C. A.: 1954. A technique for bleeding nesting birds by cardiac puncture for viral studies. Jour. Wildlife Mgt. 18:509.

Sperling, F. G., and Faber, R. F.: 1951. Wing tip amputation in a flock of wild ducks. Univ. Pa. Bul. (Vet. Ext. Quart. No. 124)

Swerbe, E. E.: 1925. Butyn in avian surgery. Vet. Med. 20:491.

Warren, D. C., and Scott, H. M.: 1935. The time factor in egg formation. Poultry Sci. 14:195.

Watel, M.: 1918. Castration chimique des coquelets par le stilboestrol. Rec. Méd. Vet. 124.412.

Wyne, J. W., McCartney, M. G., Carter, R. D., and Chamberlin, V. D.: 1959. The effect of debeaking and wing-clipping on growth and liveability of turkey poults. Poultry Sci. 38.831.

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39

Vices and Miscellaneous Conditions

Vices

Vices of poultry are those undesirable behavior patterns manifested by a few or many individuals in the flock which result in injury, death, or economic loss.

CANNIBALISM

Cannibalism occurs in various forms in all breeds of domestic fowl. According to Weaver and Bird (1934), the light breeds of the Mediterranean class are much more susceptible to these vices than the heavier breeds of the American and Asiatic classes.

Causes

Many causes of cannibalism have been suggested but often outbreaks of cannibalism occur in one pen whereas similar environmental conditions or feeding practices in other pens on the same farm do not cause any difficulty. Conditions that have been suggested as predisposing to

cannibalism are: feeding only pellets, cafeteria system of feeding, too much corn, insufficient feeder or drinker space, being without feed too long, not enough nests, nests too light, and too much light in the house (Huston *et al.*, 1956; Ostrander, 1957).

Types of Cannibalism

Picking of the vent or the region of the abdomen several inches directly below the vent is the severest form of cannibalism. This condition is generally observed in pullet flocks in high production. Predisposing factors are prolapsus of the oviduct or tearing of the tissues by passage of an abnormally large egg. Once birds acquire the taste for blood they will continue their cannibalistic habits without provocation. Many poultrymen will pick up dead birds day after day without observing that they have been picked about the vent and in some instances eviscerated.

Feather pulling is most frequently ob-

served in flocks kept in close confinement with lack of sufficient exercise. Nutritional and mineral deficiencies may be contributing factors. Irritation caused by lice and mites may induce feather pulling (see External Parasites).

Toe picking is most commonly seen among chicks. This generally starts because the chicks cannot find feed either because the hoppers are too high, they are too far from the source of heat, or there is insufficient feeder space. It is a common practice to start chicks on paper for the first few days so that they will not eat the litter. This means that there is nothing for the chick to peck at except his neighbor's toes if feed is unavailable. It is a wise practice to put mash on newspapers or on chick box covers and place them under the hover during the first few days of brooding.

Head picking usually follows injuries to the comb or wattles caused by freezing or fighting among males. A different form of cannibalism is now being observed in debeaked birds kept in cages. In this type the area about the eyes is black and blue with subcutaneous hemorrhage, the wattles are dark and swollen with extravasated blood, and the ear lobes are black and necrotic (Fig. 39.1). Even though the birds

are debeaked and kept in separate cages they will reach through the wire and peck at the neighboring bird or they will grasp the ear lobes or wattles of the bird and shake their heads in much the same fashion as a terrier shaking a rat (Angstrom, 1962).

Wing and tail picking frequently follow feather pulling or external injuries. Pin feathers are highly vascular and bleeding follows their removal or breakage. Birds will exsanguinate themselves by continual picking at a feather follicle that oozes blood or by pecking at a small lesion on the foot or other part of the body.

An unusual form of cannibalism was reported in quail by Bass (1939). It has been termed "nose-picking" as the birds peck at the top of the nose where the fleshy portion merges with the beak. The condition is generally seen in birds 2 to 7 weeks of age kept under crowded conditions. If the picking is severe enough the bird will die as the result of blood loss. If the bird survives, the beak will be permanently deformed and the males will be unsatisfactory for breeding stock. This vice occurs only when birds are brooded under artificial conditions. It seldom develops in large pens on the ground in which there is opportunity to pick and scratch. It was observed that the addition of raw
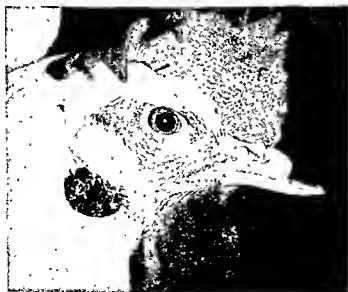
FIG. 39.1 — Cannibalism. This bird was a victim of its pen mates even though they were debeaked. Note black and necrotic ear lobe and blackening around eye and wattles due to subcutaneous hemorrhage.

meat to the ration was very effective in preventing and controlling outbreaks. It may be necessary to withhold the grain ration for a few days until the birds become accustomed to the meat.

Control

In the past, many remedies have been used to stop cannibalism such as hanging cabbages or sugar beets in the pen, putting pine boughs on the floor, painting the windows red, using a red light bulb, darkening the pen and nests, applying pine tar to picked birds, using no-pick salves, using repellent sprays, adding additional salt to the feed or water, and feeding oats. Miller and Bearse (1937) reported that an increase in fibre content of the ration reduced the incidence of cannibalism. Kennard and Chamberlin (1936) reported that feather pulling and other vices in confined birds largely disappeared when the ration was supplemented with oats. Kull (1913) found that cannibalism and feather picking were stopped by the addition of manganese sulfate and horn meal to the mash. Nelson (1956) reported effective results in 21 to 48 hours in many instances by the addition of several vitamin preparations to the feed. Willimon and Morgan (1955) reported that the addition of minor nutrient mineral elements to the ration did not show any consistent effect on feather pulling and cannibalism under experimental conditions. Neal (1956) reported that DL-methionine was effective in controlling cannibalism in laying hens, but Creek and Dendy (1957) claimed their investigations did not indicate that methionine counteracted cannibalistic tendencies.

When cannibalism has become established in a flock the only method to stop it is to apply mechanical devices such as specs or pickguards, or by debeaking. The mechanical devices have several disadvantages because they can only be put on birds of pullet size or larger, they are relatively expensive, and it takes considerable time to put them on. They do have an advantage since the operation needs to be done only once whereas it may be necessary to debeak several times.

Today the most widely accepted means of preventing and stopping cannibalism is debeaking. Debeaking can be done on birds of any age from one day to maturity. Ostrander (1957) described and illustrated in detail the various debeaking techniques. Many broiler growers and some egg producers have their chicks debeaked at one day of age (Lonsdale et al., 1957; Morgan, 1957). Care must be used in debeaking chicks to prevent adverse effects. If the beak is not cut squarely across many chicks will develop cross beaks. About one-third of the upper beak and just the tip of the lower beak are removed. Care should be observed in cauterizing the beak of chicks. Too little cauterization results in excessive bleeding and too much causes necrosis of the tissues. The incidence of "starve-outs" (failure to eat) is much higher in debeaked chicks. Therefore, every effort should be made to have the feed and water readily available for the first few days.

Many poultrymen debeak their laying stock before housing time. This is particularly true where they are kept under close confinement in colony cages. The upper beak is cut and cauterized with the electric debeaker midway between the point of the beak and the nostrils. Some also advocate that the lower beak be cut. If debeaking is done on a flock in production because of an emergency, it is best to debeak only to the "quick." This will usually stop cannibalism but will not upset production. If an electric debeaker is not available this temporary form of debeaking can be done by using a sharp jackknife. A nick is made in the beak about ¼ of an inch from the tip and with the thumb holding the cut portion of the beak against the blade the nick is rolled around the tip of the beak tearing off the horny portion and exposing the "quick." If properly done there is little or no bleeding following debeaking. If birds are debeaked severely the lower beak may grow very
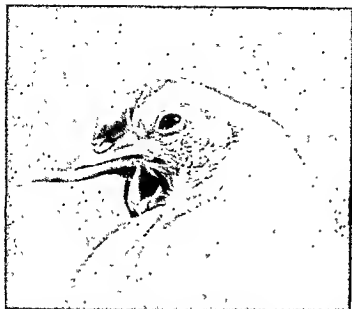
FIG. 39.2—Debeaking. Note how the lower beak has become excessively long following debeaking of the upper beak. This interferes with eating and drinking.

long in time and interfere with eating and drinking (Fig. 39.2). If this occurs it may be necessary to cut off the lower beak to permit the bird to eat and drink properly.

EGG EATING

This costly vice of chickens is similar in some respects to cannibalism in that once a few individuals acquire the habit it quickly spreads throughout the flock. Predisposing factors to egg eating are conditions which favor egg breakage such as inadequate nesting facilities, failure to collect eggs frequently, insufficient nesting material, and soft-shelled or thin-shelled eggs. If a large number of soft-shelled or thin-shelled eggs are being laid, then conditions that may be responsible for these abnormal eggs should be investigated (see the section on abnormal eggs).

The prevention of this vice is best accomplished by eliminating those conditions which favor egg breakage. Stopping the egg-eating habit is very difficult. If the birds have not been debeaked, then debeaking should be done. Once birds have developed a liking for eggs they will not only eat broken eggs but will deliberately eat any egg whether or not it has an intact

shell. Debeaking will make the end of the beak sensitive for a few days and the birds will be reluctant to peck against a hard shell. Darkening the nests by putting bags over the front or end of the nests will aid in stopping the habit. The frequent collection of eggs is mandatory if the egg-eating habit is to be checked.

HIDING OF EGGS

The primitive instinct of wild birds to lay a clutch of eggs or "steal a nest" is often manifested in all breeds of domestic poultry. It happens less frequently with chickens than with other fowl. In commercial flocks the practice rarely occurs, but in the small barnyard flocks, nests are frequently made in the haymow, hay fields, hay stacks, under buildings or woodpiles, or in various outbuildings such as tool sheds. With ducks and geese the practice most commonly occurs in the spring and seems to be a manifestation of the maternal instinct. Confinement of the birds and provision of nests will discourage the habit. The nests should be provided with clean shavings or straw and placed in a secluded spot where they will not receive too much light and should be kept free of mites and

tality from the heat are constant problems, the installation of foggers and sprinklers may be necessary.

Physical Injury

Mechanical feeder trauma. In chicks an unusual injury of the toes is caused by mechanical feeder trauma. This has been observed in flocks where there is no wire guard over the metal thrust rod that pushes against the chain link in the feed trough. The motor operating the movement of the chain runs intermittently and the chicks develop a conditioned response to the sound of the motor as they know fresh feed will enter the beginning of the trough when the motor starts. As a consequence, the chicks crowd around the beginning of the feeder and some of them have their toes crushed between the thrust bar and chain link. There is little or no bleeding associated with the injury and scab formation is followed by gangrene, necrosis, and sloughing of the toe (Fig. 39.3).

Emphysema. Subcutaneous emphysema is caused by an injury or defect in the respiratory tract that permits the accumulation of air beneath the skin (Fig. 39.4). This condition has been observed following rough handling and caponizing. After the caponizing operation the skin incision
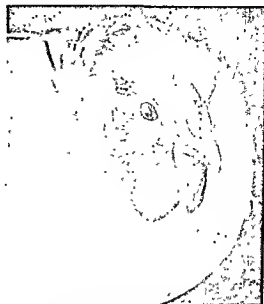
FIG. 39.4 — Subcutaneous emphysema. Note ballooning of the skin over the crop, neck, and head region.

may heal before the opening in the body wall with a subsequent accumulation of air beneath the skin. This condition, commonly called a "windpuff" can be alleviated by puncturing the skin with a sharp instrument. In aquatic or flying birds some of the pneumatic bones as the humerus, coracoid, and sternum, may fracture, allowing air to accumulate beneath the skin.

Sternal lesions. Keel bursitis occurs most commonly in *heavy, rapidly growing birds.* Pressure of the keel on the roost causes irritation and inflammation followed by callus formation or infection of the bursa. The accumulation of cheesy exudate caused by the infection produces a blemish resulting in down-grading of the carcass. Van Ness (1946) reported the isolation of *Staphylococcus citreus* from the livers, joints, and sternal bursae of birds with keel bursitis. In this case the birds were injuring the skin on sharp pieces of wire protruding from the floor of the porch. Infection developed at the site of injury and metastasized to various parts of the body. Losses ceased after the sharp ends

FIG. 39.3 — Necrosis and gangrene of the toes in a chick as the result of crushing in a mechanical feeder. (Courtesy P. P. Levine, Dept. Avian Dis., Cornell Univ.)

FIG. 39.5 — Curvature of the sternum in an adult White Leghorn.

of wire were covered. Curvature or deformity of the sternum is also associated with certain strains and breeds. Nutrition and management may play a role in the development of this condition (Fig. 39.5).

Improper sexing technique. Levine (1952) reported finding ascites, nephritis, and mortality in chicks resulting from physical injury during sexing. Affected chicks were inactive, the skin on the medial aspect of the shanks was wrinkled, and the beaks were bluish in color. These chicks died within 24 to 48 hours. The abdomen was distended with a cloudy but odorless fluid. Marked nephritis was present. Practically all of the chicks had ruptured yolk sacs. In addition there was an ecchymotic, hemorrhagic area in the inner musculature of the pelvis adjacent to the cloaca. It was found that the output of one Japanese sexer suffered a 15 per cent mortality. Losses did not occur in chicks sexed by other workers or in unsexed chicks.

Rupture of the gastrocnemius tendon. An unusual lameness of chickens described

as rupture of the gastrocnemius tendon was first reported in the United States by Bullis and Van Roekel (1944). Subsequent reports were made in Scotland by Harris (1947), in Canada by Chute (1950a and b), and by Jordan (1955) and Carnaghan (1958) in England.

The condition is characterized by an acute lameness of one or both legs. If both legs are affected the bird will rest on its hocks with the toes flexed (Fig. 39.6). At the onset of the condition, affected birds are in good condition, alert, and may even be in production. Harris (1947) indicated that birds three to nine months of age were affected. Bullis and Van Roekel (1944) and Chute (1950a and b) observed that the disease occurred most frequently in birds 4 to 7 months of age which were just starting to lay. The same investigators noted that the fall-hatched chicks had a higher incidence than chicks hatched in the spring.

Bullis and Van Roekel (1944) observed the condition only in females whereas Harris (1947) and Chute (1950a and b) re-

FIG. 39.6 — Ruptured tendons. White Rock unable to stand because of bilateral ruptured gastrocnemius tendons.

ported that males, capons, and pullets were affected. It was noted by Carnaghan (1958) that females were affected at an earlier age than males and this tended to coincide with sexual maturity. Ruptured tendons have been reported in light breeds, heavy breeds, and crosses among heavy breeds.

Harris (1947) reported a flock incidence of 3.8 per cent but Bullis and Van Roekel (1944) reported an incidence of 10 per cent and Chute (1950a and b) reported 15 per cent affected in one flock. In one experiment where the offspring were derived from previously affected parents, Carnaghan (1958) observed 20 per cent of the flock became affected.

Externally the lesion is manifested by a bluish green discoloration of the skin above the hock. It is often possible to palpate a break in the gastrocnemius tendon in the live bird. Fibrosis and thickening occurs

around the tendon in old lesions and indurated areas can be palpated through the skin. The gastrocnemius tendon may have a complete transverse break (Fig. 39.7) or the tendon may have a grey water-soaked appearance. Hemorrhage into the affected area may be slight or extensive.

On histological examination, Harris (1947) noted round cell infiltration in the grossly thickened tendons but none where complete rupture without fibrosis had occurred. Chute (1950a and b) found that muscle fibers were degenerated and some had disappeared adjacent to its tendinous attachment. There was hyaline degeneration of the tendon. Adjacent to this area was a network of collagen which surrounded lacunae containing individual, variously shaped darkly blue staining cells. Proliferating fibroblasts were present which constituted the thickening about the tendon. Upon examination of blood from
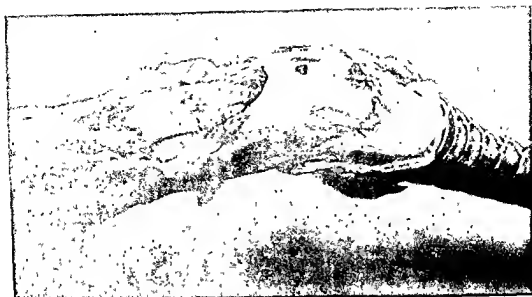
FIG. 39.7 — Ruptured tendon. Skin reflected from the hock joint revealing the ruptured and hemorrhagic ends of the gastrocnemius tendon

affected birds, Chute (1950a and b) did not find any abnormalities in the hemoglobin content, red cell count, white cell count, and differential count.

The exact cause of ruptured tendon is unknown. Bullis and Van Roekel (1944) observed the condition in the spring in birds hatched out of season. He suggested that the tendons may lack strength and are predisposed to rupture under the strain of jumping from roosts, nests, or feeders. Chute (1950a and b) failed to find any bacterial agent using aerobic and anaerobic techniques. He discussed three theories as to the possible etiology—nutrition and management, genetics, and predation, but drew no conclusions. The results of Carnaghan's experiment (1958) suggest that a susceptibility to the condition may be inherited. Jordan (1955) was of the belief that genetic factors were concerned in the etiology, although he believed that out-of-season hatching, environmental conditions, and early maturity were contributing factors.

Cranial injury. Injury to the skull and brain is most commonly seen in quail, pheasants, and pet birds such as canaries and parakeets. Quail and pheasant take off from the ground with great thrust and will fly directly into objects under the stress of fear. The force of the impact will cause hemorrhage in the cancellous bone of the skull, and intracranial hemorrhage. Sudden death in a quail or pheasant in excellent physical condition with no evidence of internal lesions or infectious disease should prompt one to examine the skull for injury. Pet birds that escape from their cages or are released for exercise in the house will frequently fly against windows or walls and sustain cerebral injury. The bird does not die immediately but generally succumbs within a period of hours following the accident. The bird may act sleepy or manifest nervous symptoms prior to death. A history of acute death in a well-nourished pet bird with no obvious sign of disease should lead one to investigate the possibility of cranial injury.

Smothering. This condition is generally caused by birds crowding or piling up in a corner. It may occur when birds are moved to new quarters, when they are frightened, or in young birds when they are chilled. The history of the case generally indicates that mortality occurs only at night and the flock in general looks healthy. Smothering of baby chicks can occur in chick

boxes that are piled too high without an air space between each box, or in boxes that do not have sufficient ventilation holes and in boxes that are placed in a closed compartment such as the trunk of a car. Postmortem examination of chicks that have smothered usually does not reveal enough gross pathology to make a positive diagnosis but will aid in the elimination of other possible causes of death. In broilers and older birds that have smothered there is congestion of the trachea and lungs, and the feathers will be worn off the birds where they have been trampled. Smothering of chicks in the brooder house can be controlled by putting a circle of corrugated cardboard around the hover for the first week and gradually widening its diameter as the chicks get older. This will prevent the chicks from piling up in a corner during the night. When birds are moved to new quarters the use of a dim light or lantern the first few nights will decrease the possibility of smothering. Birds transferred to new quarters should be checked late in the evening for signs of piling. Care is important the first few days after acquiring a batch of new chicks or grown birds.

Dehydration. Dehydration can occur in birds of any age. It is generally caused by failure of the birds to find the water, inability to reach the water, and in some cases by a deterring factor in the water. Baby chicks can survive several days without water but will start to die by the fourth and fifth day if they have not taken any water. The mortality will reach its peak during the fifth and sixth days and terminate abruptly thereafter. The chicks that are not drinking will have succumbed by this period and the survivors are those that have found the water and are drinking. Dehydration can be detected in a chick as it is unable to "peep" during the later stages. The chick lacks sufficient weight for its size and age, and the skin on the medial aspect of the shanks is dehydrated and folded. Other changes in a dehydrated chick are a blue discoloration of the beak, the drying and darkening of the breast musculature, darkening of the kidneys, an accumulation of urates in the ureters, and darkening of the blood. Symptoms and lesions in older birds are similar to those in chicks and the loss in weight is more noticeable. To prevent dehydration in chicks the water fountains should be placed at the edge of the hover directly upon the litter without any platform. When a change is made from a small drinker to a larger type or to automatic drinkers, the old type of drinker should be kept for a few days and moved towards the new source of water supply to gradually accustom the birds to the change. When birds are moved from range shelters to laying houses, drinking pans should be placed on the floor for the first few days until the birds become adjusted to their new environment. On occasion an electrical charge may be present in the water caused by faulty electrical heating devices used to prevent water from freezing. The drinkers will be full of water and the owner will be unaware that dehydration is occurring if the system is automatic. Even if the problem of dehydration is made known to the owner he may be unaware of the reason why the birds are not drinking unless he puts his hand in the water. On one occasion a short circuit was occurring in the laying house and the same water pipes also supplied the drinkers on range. The owner put temporary drinkers in the laying house because the birds had become conditioned to the electric shock and refused to drink from the old drinkers. However, he was unaware that the same situation occurred on the range and only after more mortality had occurred and the situation re-evaluated at the laboratory did he come to the realization that the birds on range had also been conditioned by the electric shock and would not drink from the usual pans. Laying birds need a constant water supply or production will drop. Frozen pipelines or frozen water pans are followed by dips in the production charts. Cold weather followed by a drop in production should lead to an investigation of the possibility of a frozen water supply.

Chemical Injury

Brooder stove fuel burns. Bullis and Van Roekel (1944) reported exfoliation of the cranial skin in chicks caused by contact with kerosene or fuel oil. This problem occurred where kerosene or fuel oil brooder stoves had a small leak in the fuel line which dampened the bottom of the pipe. The chicks became exposed by brushing their heads against the pipe. When the cause was explained to the flock owner he usually expressed disbelief until he ran his hand along the bottom edge of the pipe. The kerosene causes irritation and necrosis. An eschar is formed which eventually drops off leaving the surface smooth and devoid of down. The shrinkage of the skin on top of the head produces tension on the eyelids causing distortion and angulation (Fig. 39.8). This combination of bald head and angular eyelids has led to a description of the condition as "china boy" disease. The chicks rapidly recover once the source of irritation is removed.

Dickinson and Clark (1946) reported brooder stove residue burns in turkey poults. The poults came into contact with the tarlike residue that leaked from the pipes of brooder stoves burning gas briquettes. There was severe coagulation necrosis of the skin over the skull and on the back of the neck. It was discovered that exposure to sunlight was necessary for irritation and subsequent lesions to occur. Birds exposed to sunlight would rub the base of the skull and neck over the wings and back. Some birds would shake their heads so violently as to fall over. The intense irritation would cause the birds to scratch almost continually and scratches or lacerations would be produced on the head and eyelids. Birds with severe irritation would quickly get relief when removed from the sunlight. In one experiment, four 6-week-old poults were rubbed with residue on the head and neck. The birds were exposed to sunlight for 2 hours on 4 successive days. Three of the birds died on the fourth day. Four other poults treated with the same material but not exposed to sunlight developed a mild dermatitis but showed no visible signs of discomfort. The condition was readily corrected by confining affected birds for 10–14 days until the lesions healed.

Keratoconjunctivitis caused by ammonia burns. The first description of keratoconjunctivitis in poultry was given by Barber (1947) who observed this condition when birds were reared under unsanitary conditions. Bullis et al. (1950) thoroughly investigated the circumstances under which this condition appeared and drew the conclu-

FIG. 39.8 – Angular eyelids and loss of down on top of the head in the chick on the left caused by kerosene burn. Normal chick on right.
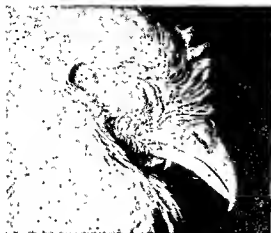
FIG. 39.9 — Photophobia in a chicken with keratoconjunctivitis caused by ammonia burn.

sion that it was caused by exposure to ammonia fumes. Experimental reproduction of the syndrome was carried out by Faddoul and Ringrose (1950). Wright and Frank (1957) recorded the condition in Canada and more recently Saunders (1958) in England described a field outbreak in broilers.

The condition is usually seen in young birds during the winter and spring months. The first symptoms manifested are a rubbing of the head and eyes against the wing. The bird remains quietly in one

spot with the eyelids closed as there is a marked photophobia (Fig. 39.9). Upon close examination of the conjunctiva, edema and inflammation are evident and the surface of the cornea may be roughened or opaque (Fig. 39.10). The center of the cornea may have a shallow ulceration with irregular edges while the remaining area of the cornea is normal in appearance. Generally the condition is bilateral but may be unilateral. In chronic cases, the outline of the eyelids is irregular. Affected birds soon become emaciated because they do not eat.

The condition is apparently caused by continued exposure to ammonia fumes generated by decomposition processes occurring in the droppings (Bullis *et al.*, 1950; Wright and Frank, 1957; Saunders, 1958).

The experimental exposure of birds to ammonia fumes by Faddoul and Ringrose (1950), Wright and Frank (1957), and Saunders (1958) resulted in the production of lesions similar to those occurring in field cases. Prevention of this condition is based on sound management practices with particular attention to ventilation, clean litter, and adequate floor space. Affected birds may take a month or longer to recover even though removed to well-
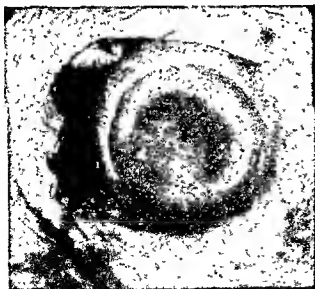
FIG 39.10 — Corneal opacity caused by ammonia burn.

FIG. 39.11 — Diphtheritic patches in the mouth of a chicken caused by *Spirillum pulli* infection.

ventilated quarters. Under commercial conditions disposal of severely affected birds is indicated.

CONDITIONS AFFECTING THE DIGESTIVE SYSTEM

Stomatitis. Mathey (1956) reported the occurrence of diphtheritic patches in the oral cavity of chickens caused by *Spirillum pulli* sp. *nova* (Fig. 39.11). The organism could be demonstrated in fresh scrapings taken from salivary glands or diphtheritic lesions and examined by dark-field illumination or in Giemsa-stained preparations.

The organism was not grown in pure culture but transmission was accomplished by experimental inoculation of tissue suspensions and by contact.

String eating. Respiratory distress caused by string looped around the base of the tongue and passing down the esophagus has been observed in chickens and turkey poults. Poults are commonly reared in batteries with crinoline cloth covering the floor. Strands of thread protrude from the cut edges and the poults ingest these fibers, some of which are looped around the tongue. Continual swallowing movements draw the string tighter until it cuts into the tissue causing edema of the glottis. Although the condition could easily be alleviated it is usually detected only at necropsy. Chickens frequently pick up the long white strings used to stitch together the tops of feed sacks. The string becomes looped around the base of the tongue (Fig. 39.12) and the bird will keep extending its head and neck in much the same manner as a bird with laryngotracheitis.

Occasionally pieces of grain or pellets become lodged in the larynx or trachea. Frequently they may be retracted through the oral cavity with a suitable instrument. Foreign bodies which cannot be reached in this manner can be removed by tracheotomy. Procaine is suitable for local anesthesia.

FIG. 39.12 — String looped around the base of the tongue and larynx of a chicken.

Beak necrosis. Beak necrosis is practically a condition of the past with our modern feeding practices. It is caused by a gradual accumulation of feed inside the mouth along the edge of the beak. Infection and necrosis gradually occur followed by sloughing of the beak. The condition is more prevalent with finely ground feeds or those that tend to paste when eaten. Supplying finely ground corn or a coarser diet usually corrects the condition.

Curled tongue condition. The first report of this condition was made by Hudson (1939) who reported on a curled tongue condition in turkey poults a few days old (Fig. 39.13). Twenty-five per cent of a flock of 200 poults were affected. The outcome or cause was not discussed.

Grau (1945) first noted that chicks fed a diet deficient in any one of three amino acids (leucine, isoleucine, and phenylalanine) developed a folded condition of the tip of the tongue (Fig. 39.14). He could cure the condition in a few days following the addition of the deficient amino acid to the diet. In the same chicks the folded tongue condition reappeared when one of the amino acids was withheld from the diet.

Sanger et al. (1953) described a curled tongue condition occurring in Broad Breasted Bronze and Beltsville White turkeys over a period of several years. In

FIG. 39.14 – Curled tongue condition in a young chick.

one case the birds received a commercial feed and in another case a home-mixed ration was fed. The condition was first noticed at 10 days of age and by 6 weeks of age 200 in a flock of 4,000 were affected (Fig. 39.15). In addition to its folded state the tongue rested in a submandibular pocket that protruded between the rami of the mandible (Fig. 39.16). Feed adhered to the floor of the mouth in the anterior portion of the intermandibular space. Surgical removal of the curled portion of the tongue did not improve the ventral displacement. Histological examination of the tongue revealed nothing unusual.

The defect in the tongue had interfered with feed intake and affected birds were below normal weight. Although the ration was changed to commercial crumbles at 6 weeks of age, the condition was not improved in affected birds but no new cases developed. It is possible that the pathological change had reached an irreversible state even though the causative factor or factors had been removed.

Scott, in 1951, quoted by Bragg (1953), found a high incidence of the curled tongue condition among poults used in an

FIG. 39.13 – Curled tongue condition in a turkey poult.

FIG. 39.15 — Curled tongue condition in a Beltsville White turkey. (Courtesy Dr. V. L. Sanger, Dept. of Veterinary Pathology, Ohio State University.)

experiment testing various high energy ingredients from carbohydrate sources. The leucine, isoleucine, and phenylalanine were calculated to be 1.3 times the known chick requirement and he concluded the curled tongue condition was not caused by an amino acid deficiency in this case but was

FIG. 39.16 — Submandibular pocket caused by ventral displacement of the tongue in a turkey with curled tongue condition. (Courtesy Dr. V. L. Sanger, Dept. of Veterinary Pathology, Ohio State University.)

probably due to a high amount of red dog flour in the ration which caused pasting in the beaks of the poults and caused the tongue to curl back mechanically. He further stated that when the level of red dog flour was reduced or replaced by cornmeal, pulverized oats, or standard middlings, no cases of curled tongue condition occurred.

Bragg (1953) conducted extensive experiments in studying the curled tongue condition in poults and he made several observations. First, that some cases of curled tongue occur at hatching time. He stated, "This may be explained by a fairly rare recessive genetic factor or a genetic difference in the nutritional requirements of the breeding hen, or the differential depletion of nutrients in a few breeder hens in the flock supplying the hatching eggs." Second, a greater incidence of the condition occurs among poults fed fine mash than among those fed a coarser type ration. Third, under normal growing conditions, it appears that the deformity is not caused by a deficiency of the amino acids—leucine, isoleucine, or phenylalanine—in the poults' diet.

Wright and Temperton (1955) in Great

Britain reported that poults of four different breeds and one cross-breed group developed typical symptoms of curled tongue when fed a home-mixed dry mash. The condition also occurred when commercial mash was fed in the dry state. From 5 to 26 per cent of the poults became affected. When the rations were fed in a moistened condition from the time of hatching, only an isolated case of curled tongue was observed. A large proportion of affected poults recovered when a change was made from dry to wet mash feeding. They concluded that the primary cause of curled tongue condition in turkey poults is the feeding of a dry mash of fine physical consistency during the first few weeks of life.

Impaction. Crop impaction occurs when large amounts of fibrous material are ingested (Fig. 39.17). This is apt to occur when birds are first put on range that has long tough grass. If birds are allowed to go without feed for any length of time and there is straw or grass available they will eat this material. In some cases where straw is used for litter, birds will eat this material even though feed is available. The fibrous material will form a ball in the crop and if not too large it will begin to pass through the remainder of the digestive tract. It may become lodged at any point along the digestive tract (Fig. 39.18). Birds with impaction may survive for days but gradually they become emaciated and die of inanition. Early detection of crop impaction can be corrected by surgery. If the condition is allowed to remain too long, atony of the muscles may occur.

Proventricular hypertrophy. A peculiar hypertrophy of the proventriculus in 4-week-old chicks fed a purified diet was reported by Newberne *et al.* (1956). Approximately 15 per cent of the chicks which originated from 3 different sources were
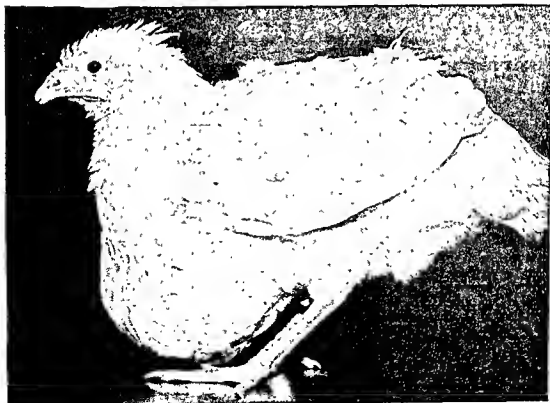
FIG. 39.17 — Marked distension of the crop caused by impaction with straw.

FIG. 39.18 — Large fibrous mass lodged in the lower end of the esophagus. Note how the pressure of the mass has flattened the primary bronchi.

FIG. 39.19 — The greatly enlarged proventriculus on the right may be of dietary origin.

Normal
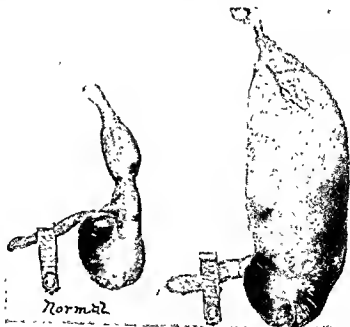
affected. It was suggested that the condition may have been dietary in origin (Fig. 39.19). The author has also noted unusual enlargement of the proventriculus in chicks fed a commercial ration.

Traumatic ventriculitis. Traumatic ventriculitis is generally caused by a sharp object such as a nail, wire, or stick perforating the wall of the gizzard. Contraction of the powerful gizzard muscle forces the object through the wall (Fig. 39.20). Birds with this condition become emaciated and death follows. Adhesions and inflammatory exudate mark the site of injury (Fig. 39.21).

Intussusception. Intussusception rarely occurs in the fowl. The invagination of the upper portion of the intestine into the lower portion with subsequent circulatory arrest and adhesions of the involved parts makes the diagnosis obvious. The cause is not always apparent but cases have been observed where the birds were affected with ulcerative enteritis and coccidiosis (Fig. 39.22). Birds may show signs of illness for several days before death. If an early diagnosis were made, resection of the affected intestine could be performed.

Typhylitis. Mathey and Zander (1955) described a typhylitis of chickens, turkeys, and pheasants characterized by caseous granulomas (Figs. 39.23 and 39.24). A spirochete which they believed was *Spironema ceci-gallorum* was isolated from the lesions (Fig. 39.25). The organism could not be grown in artificial media but was grown in embryonating eggs. The feeding of cecal tissues containing spirochetes to one-week-old chicks produced cecal nodules 30 days postinoculation. Spirochetes were demonstrated in the lesions of the inoculated chicks. Lesions were not produced with pure cultures.

Anomaly. Occasionally an anomaly of the cecum is encountered in which only one cecum is present with a bifurcation forming two cul-de-sacs at its blind end (Fig. 39.26).

CONDITIONS AFFECTING THE SKIN AND INTEGUMENT

Stunted chick disease. During the decade between 1943 and 1953 a condition in chicks characterized by stunting and mortality during the first weeks of life was of common occurrence. In subsequent years the number of cases has declined according to diagnostic laboratory reports. Typical symptoms reported by Robertson *et al.* (1949) are rough feathering with brittle

FIG. 39.20 — Traumatic ventriculitis in a pheasant. A sharp pointed stick has been forced through the gizzard muscle.

FIG. 39.21 — Traumatic ventriculitis in a pheasant. A sharp pointed stick (arrow) has been forced through the gizzard muscle and has eroded a hole through the sternal muscles. Note inflammatory exudate surrounding the injured area.

FIG. 39.22 — Intussusception. Note the congestion caused by circulatory arrest at the origin of the invagination. Lesions of ulcerative enteritis can be seen in other portions of the intestinal wall.
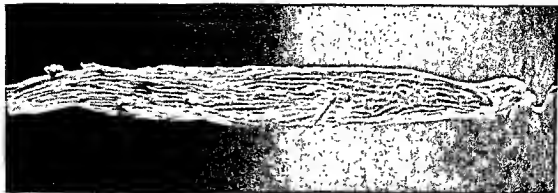
FIG. 39.23 — Turkey cecum with small nodules on the mucosal surface in typhylitis. (Courtesy Dr. W. J. Mathey, Jr., Dept. of Veterinary Microbiology, Washington State Univ.)
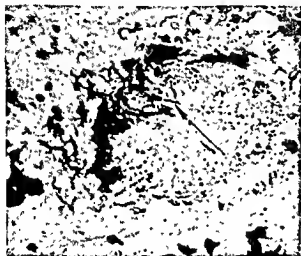
FIG. 39.24 — Histological section of a nodule in a turkey cecum containing many spirochetes. Levaditi stain. X800. (Courtesy Dr. W. J. Mathey, Jr., Dept. of Veterinary Microbiology, Washington State Univ.)

FIG. 39.25 — Spirochetes in a smear taken from a cecal nodule. X1350. (Courtesy Dr. W. J. Mathey, Jr., Dept. of Veterinary Microbiology, Washington State Univ.)
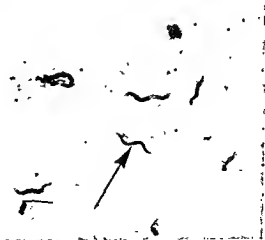
FIG. 39.26 — Cecal anomaly in a chicken. Only one cecum present with a bifurcation at its tip.

and broken primary and secondary wing feathers producing a characteristic ragged appearance. Some chicks have encrustations at the commissures of the mouth and granulations on the eyelids causing them to adhere. Growth is severely depressed (Fig. 39.27). Mortality progressively increases to a peak ranging from 25–75 per cent at about the fourth week. Survivors at 5–6 weeks of age recover and grow normally and at maturity show no ill effects from their earlier condition. Field trials were conducted by Robertson *et al.* (1949) in an effort to find the cause of this condition.

The addition of 5 per cent liver meal or 5 per cent dried brewer's yeast to the ration improved weight gains but did not prevent the condition. Once the condition became established, the injection of 100 micrograms of pantothenic acid or riboflavin had little effect although growth was slightly improved. It was concluded from their field trials that neither feed nor source of chicks appeared to be predisposing factors in the production of stunted chick disease. Angstrom (1962) believed that the problem may have been the result of several factors, primarily mismanagement, rather than a single etiological factor.

FIG. 39.27 — Stunted chick disease. Ragged feathering, granulations on the eyelids and encrustations at the commissures of the mouth in a 19-day-old chick. (Courtesy Dr. P. P. Levine, Dept. of Avian Dis., Cornell Univ.)

of *Ammi visnaga* seeds and subsequent exposure to sunlight caused vesicular dermatitis in chickens and ducks in 4 to 7 days. Birds fed *Ammi visnaga* seeds and shielded from the sun did not develop dermatitis. The lesions and course of the disease were similar to that described by Hoffman (1939) and Perek (1958). Staphylococci and other bacteria were isolated from the vesicles. Subcutaneous and intramuscular inoculations of these organisms produced no visible change. Scab suspensions swabbed on scarified areas on the comb and wattles produced no effect. Trenchi (1962) reported on comparative experimental studies of the lesions produced by ergot poisoning and vesicular dermatitis produced by photosensitization after the ingestion of *Ammi visnaga* seeds. He stated that vesicles were not produced by ergot poisoning and that it took two weeks for lesions to appear in ergot poisoning.

In summary, reports have indicated that vesicular dermatitis may be caused by micrococci, *Lolium temulentum* seeds contaminated with *Cladosporium herbarum*, and photosensitization by ingestion of *Ammi visnaga* seeds and subsequent exposure to sunlight.

Xanthomatosis. This unusual skin condition has been reported in chickens from numerous areas in the United States and from Belgium by Thoonen *et al.* (1959). Hudson (1953) observed three flocks of White Leghorns affected with swollen wattles and cutaneous swellings. Clinical cases and investigational studies were reported by Peckham (1955), Corner *et al.* (1959), Greve and Moses (1961), and Meinecke *et al.* (1962).

The condition has been observed primarily in White Leghorns of varying genetic background. Lesions usually become evident as birds approach maturity at 6–7

FIG. 39.29 — Swollen wattles and cystic swelling on the breast of a bird with xanthomatosis. (Courtesy Dr. P. P. Levine, Dept of Avian Dis., Cornell University.)
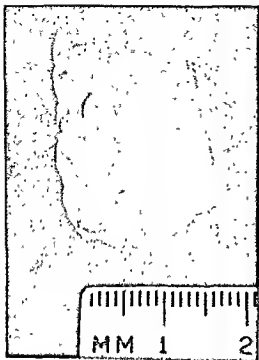
FIG. 39.31 — Xanthomatosis. Cross section of thickened abdominal skin from bird in Fig. 39.30. (Courtesy P. P. Levine, Dept. of Avian Dis., Cornell Univ.)

FIG. 39.32 — Nodular swellings on the abdomen and thighs of a chicken with xanthomatosis.

histopathologic changes occurring in xanthomatosis. The microscopic lesions vary but all have similarities depending upon the stage of the lesion. Early lesions are infiltrated with vacuolated lipoid-laden macrophages commonly called "foam cells" (Fig. 39.33). Lymphocytes, occurring singly or in clumps, are characteristic and often numerous. A striking feature is the presence of lenticular spaces or clefts produced by cholesterol deposits in the tissue (Fig. 39.34). Multinucleated giant cells may surround the cholesterol crystals. Frozen sections when viewed with polarized light reveal birefringent, rhombic crystals (Fig. 39.35) and the typical "maltese cross" effect of cholesterol esters (Fig. 39.36). Schultze's histochemical test for cholesterol and related substances is positive on frozen skin sections. Cholesterol determinations on the affected skin show a marked increase over normal. Blood cholesterol determinations are normal.

The skin lesions are permanent and affected birds are unfit for meat consumption. Control measures are lacking until more is learned regarding the etiology of this condition.

Bumblefoot. This term is used to designate a localized infection in the foot causing bulbous swelling of the foot pad and surrounding tissues. The condition may be unilateral or bilateral. Generally only a few individuals in the flock are affected but in some cases it is widespread throughout the flock. Infection is believed to occur by means of an injury to the ball of the foot. As the infection progresses, the lesion enlarges and the tissue between the toes become greatly distended (Fig. 39.37). Eventually the swelling will ulcerate through the plantar surface (Fig. 39.38). The birds be-
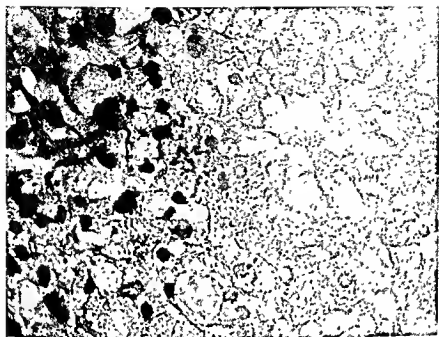
FIG. 39.33—Histological section of xanthomatous lesion illustrating the vacuolated structure of the cytoplasm in the foam cells. ×470. (AFIP 54-7256.)

FIG. 39.34—Histological section of xanthomatous skin showing lenticular spaces produced by deposits of cholesterol crystals. ×470. (AFIP 54-5394.)

FIG. 39.35 — A frozen, unstained, histological section of a xanthomatous lesion as seen by polarized light. The birefringent property of the cholesterol crystals (white areas) is contrasted against the black background of the tissue. ×100. (Courtesy of J. H. Greve and H. E. Moses, Dept. of Veterinary Science, Purdue Univ.)
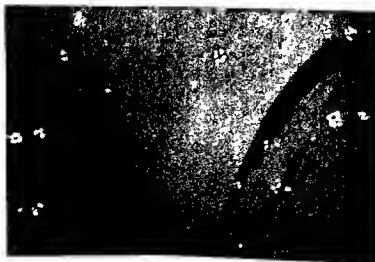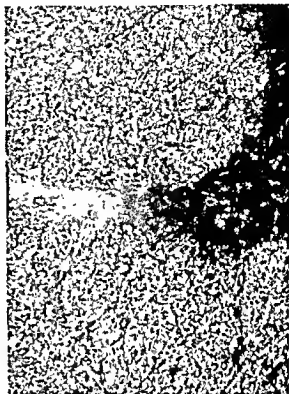
FIG. 39.36 — A frozen, unstained, histological section of a xanthoma viewed by polarized light. The birefringent, "maltese cross" effect of cholesterol esters is visible against the black background. ×400. (Courtesy of J. H. Greve and H. E. Moses, Dept. of Veterinary Science, Purdue Univ.)
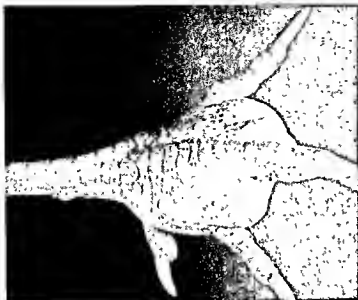
FIG. 39.37 — Bumblefoot. Marked swelling of the foot pad and surrounding tissues in a chicken.

come lame, have a diminished appetite, and stop laying. Staphylococci may be isolated from the lesion and where ulceration has occurred, mixed infections will be encountered. If treated early, surgery and antibiotic therapy may alleviate the condition. In commercial flocks, disposal of the affected bird is generally indicated.

Plantar necrosis. Angstrom (1961) observed a condition in adult chickens characterized by necrosis and exfoliation of the skin on the plantar surface of the feet (Fig. 39.39). The litter in the pens was wet and filthy and masses of fecal material were caked on the feet. The hardened masses of material on the feet did not allow for normal exfoliation of the epithelial cells and the accumulation of dead tissue and moisture provided a favorable site for growth of organisms of the necrophorus type. When litter conditions were improved the problem stopped.

Abscess of the uropygial gland. The uropygial gland is a sebaceous gland lo-

FIG. 39.38 — Bumblefoot in a turkey with ulceration of the plantar surface.

FIG. 39 39 – Plantar necrosis. Necrosis and exfoliation of the skin on the foot pad and toes of a chicken.

cated on the back of the bird at the base of the tail. Infection of the gland or obstruction of the excretory duct may cause an accumulation of cheesy exudate. When this happens, the area surrounding the gland becomes inflamed and swollen. Incising the area, removing the exudate, and applying an antibiotic will generally cure the condition.

DISEASES OF THE CIRCULATORY SYSTEM

Round heart disease. Matzke (1942) in Germany first reported on "Eierherzen" (egg heart) in chickens. Fischel (1946) reported on cases of "toxic heart degeneration" which he had observed in New Zealand as early as 1941. Luke (1917) in Australia described a similar disease in fowls under the name of "round heart disease." According to Adsersen (1948) the disease has been prevalent in Denmark since 1936 where it is described by the term "yellow heart degeneration." Since these early reports, numerous workers have recorded observing this condition in many countries including the United States.

The disease affects both males and females and manifests an acute or subacute course. Most losses occur following exertion by the birds, and death often occurs in the presence of the owner at feeding time. The birds topple over or fall off the perches and kick aimlessly for a few minutes. In some birds there is a short period of inappetence and cyanosis of the comb before death. Adsersen (1948) reported the highest incidence occurred in Brown Leghorns. Wilson (1957) also noted that the condition occurred most frequently in Leghorn types but had seen Rhode Island Reds affected. Levine (1958) reported two outbreaks in White Leghorns.

Most observers have recorded a seasonal incidence of the disease with the greatest number of cases occurring during the fall and winter months. Levine (1958) however recorded the heaviest losses during July. Mortality is variable but may reach 50 per cent and higher over a period of several months (Fischel, 1946). Pullets are most commonly affected but Wilson (1957) reported having seen the disease in birds varying in age from 8 weeks to over a year.

On postmortem examination the birds are well fleshed with a marked congestion of the venous system. The pericardial sac may be distended with fluid or yellow gelatinous transudate. The heart is enlarged and has a blunt apex that may have whorled indentation at its apex (Fig. 39.40). The myocardium has a parboiled or yellowish appearance with linear streaks running parallel with the muscle fibers. Ascites may be accompanied by a layer of gefatinous transudate over the liver surface.

On histological examination of the heart, Fischel (1946) reported there was a loss of cross striations and pyknosis and karyolysis of nuclei. The muscle fibers had lost their eosinophilic property and there was evidence of granular disintegration. Adsersen (1948) reported fatty infiltration of the myocardium. Wilson and Siller (1954) and Iyer et al. (1959) reported the presence of intranuclear inclusions in myocardial fibers.

The etiology of round heart disease is unknown at the present time. All attempts to incriminate bacterial, viral, fungal, or

FIG. 39.40 — "Round heart" on the right with enlarged and dimpled apex. Normal heart on the left.

toxic agents have failed. Nutritional studies have met with failure. The role of genetics needs further investigation. Wilson (1958) and Levine (1958) have noted that most outbreaks occur on built-up litter, and when survivors of an outbreak were transferred to clean litter they showed a striking and almost immediate improvement. Wilson also demonstrated in two experiments that the disease can be induced in pullets by running them on litter previously used by affected birds.

Hemorrhagic syndrome. In the past decade a condition characterized by hemorrhages and mortality has been seen in chickens with increasing frequency. The first report on this condition was by Baker and Jaquette (1953) who recorded their observations concerning a "hemorrhagic syndrome" in poultry. They indicated that for the 3 years preceding their report an increasing number of birds were submitted to the diagnostic laboratory with unexplainable hemorrhages occurring in various tissues. Goldhaft and Wernicoff (1954) described a similar pathological syndrome and indicated that they had seen the disease as early as 1951.

Clinical signs. The disease has been seen in birds ranging in age from 5–15 weeks with most cases occurring between 5 and 9 weeks of age. Mortality is variable, ranging from a low of 1 per cent to a high of 40 per cent with an average of 5–10 per cent (Baker and Jaquette, 1953; Gray et al., 1954; Goldhaft and Wernicoff, 1954).

Signs manifested by affected birds are paleness or icteric discoloration of the tissues about the head. Hemorrhage may be noted in the anterior chamber of the eye (Fig. 39.41). The feathers are ruffled, the birds act droopy and have a tendency to huddle. A diarrhea has been noted in some cases (Gray et al., 1954; Cover et al., 1955). Washko and Mushett (1955) indicated that the course of the disease was usually about 3 weeks and in many flocks the prime cause of economic losses was decreased feed consumption and poor feed conversion with a resultant delay in marketing.

Gross lesions. At necropsy characteristic findings are hemorrhages in the musculature and viscera. The blood may have a pale, watery appearance. An occasional bird may have hydropericardium (Fig. 39.42). The most constant lesion and of great aid in making a diagnosis, if exten-
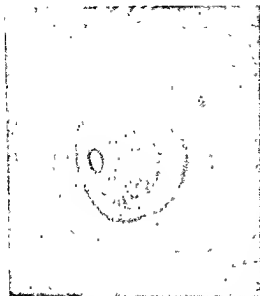
FIG. 39.41 — Hemorrhage in the anterior chamber of the eye in a chicken with hemorrhagic disease.

sive hemorrhagic changes have not occurred, is a pale and fatty bone marrow (Fig. 39.43). This change in the appearance of the bone marrow is due to a decrease in hematopoietic elements which are replaced by fatty tissue (Gray *et al.*, 1954). Irregular, scattered hemorrhages may be

present in the breast and thigh muscles (Figs. 39.44 and 39.45). Punctate hemorrhages may be found in the mucosa of the proventriculus at its junction with the gizzard, and hemorrhage may occur beneath the gizzard lining causing blackening and sloughing (Fig. 39.46). In addition, focal hemorrhages may be found in the wall of the crop, and "paint-brush" splotches may occur in the myocardium. The intestine may have punctate hemorrhages in the mucosal and serosal surfaces and on occasion a bloody core is present in the cecum (Fig. 39.47). The presence of blood in the cecum necessitates a differential diagnosis from cecal coccidiosis. A microscopic examination of the cecal contents together with a careful evaluation of the history and other clinical findings should aid in arriving at a diagnosis. Gray *et al.* (1954) reported that subcutaneous hemorrhage of the shanks and feet frequently resulted in the formation of ulcers. Hemorrhages may be present in the liver, spleen, and kidney. The liver may be yellow in color with pinpoint hemorrhages scattered throughout the parenchyma or it may present a reticulated network particularly along the edges (Marthedal and Velling, 1961) (Fig. 39.48). Occasionally a yellow gelatinous transudate

FIG. 39.42 — Hydropericardium associated with hemorrhagic disease.
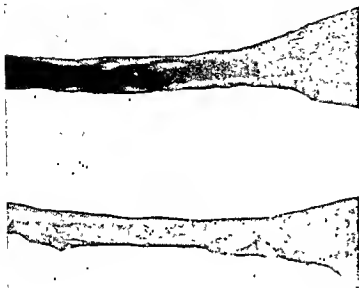
FIG. 39.43 — Hemorrhagic disease. Pale aplastic marrow in tibia from a chicken with hemorrhagic disease (bottom) contrasted with the dark red marrow in the tibia from a normal bird (top).

may be noted in the subcutis of the neck, breast, and thighs (Hanley, 1962). Baker and Jaquette (1953) reported that the affected birds may have nephritis. Marthedal and Velling (1961) noted hemorrhages and fungal granulomas in the lungs which they regarded as a manifestation of reduced resistance. Gray et al. (1954) noted liver necrosis and intestinal ulcers associated secondarily with the disease during the terminal stages.

Hematology. Gray et al. (1954) observed leukopenia and anemia associated with depressed bone marrow activity. The anemia was the normocytic, normochromic type. Abnormal thrombocytes were consistently observed on blood smears. These cells were enlarged, more circular than normal, and highly vacuolated. Cover et al. (1955) observed similar blood changes and noted that the prothrombin time was never prolonged. Hanley (1962) confirmed the results of earlier investigators and noted that the blood picture showed a reduction in red blood cells, granulocytes, and thrombocytes.

FIG. 39.44 — Hemorrhages in the breast muscle of chicken with hemorrhagic disease.

FIG. 39.45 — Hemorrhages in the thigh and leg muscles of a chicken with hemorrhagic disease.

Histology. Gray *et al.* (1951) reported that the marrow of affected birds was devoid of hematopoietic elements and was replaced with fatty tissue. Most of the sinusoids were collapsed. Hypoplastic bone marrow showed conspicuous reduction of myelocytic elements. In extreme cases, only sinusoidal endothelial cells, interstitial reticular cells, and fat cells were present. A few lymphocytic loci were seen. Cover *et al.* (1955) noted varying degrees of hemorrhage and necrosis in the liver. The parenchyma adjacent to the surface was

most frequently involved. Vessels in the affected area appeared congested and bile stasis was evident. The lymphoid nodules of the spleen had indistinct borders and appeared hypoplastic. Irregular areas of hemorrhage were seen in the red pulp. Hyalinized material was common in the adenoid sheaths and lymphoid nodules. The kidney showed evidence of coagulation necrosis in the tubular epithelium, and infiltration of lymphocytes was frequently seen.

Etiology. There is a lack of general

FIG. 39.46 — Hemorrhagic disease. Hemorrhage at the junction of the gizzard and proventriculus.

FIG. 39.47 — Punctate hemorrhages in the intestine of 6-week-old chick with hemorrhagic disease. (Courtesy Dr. P. P. Levine, Dept. of Avian Dis., Cornell Univ.)

FIG. 39.48 — Hemorrhages in the heart and liver of a chicken resulting from hemorrhagic disease.

agreement among the investigators as to the etiology of hemorrhagic syndrome. The pathological and hematological changes of aplastic anemia in cattle caused by feeding trichloroethylene-extracted soybean oil meal are similar to those encountered in chickens affected with hemorrhagic syndrome, and this prompted investigation into the role of trichloroethylene-extracted soybean oil meal in the hemorrhagic syndrome. Pritchard et al. (1952) and Sautter et al. (1952) reported that chickens were unaffected by eating soybean oil meal that was toxic for cattle. Eveleth and Goldsby (1953) reported mortality, retarded growth, pliable bones, and lowered resistance to disease in chicks fed experimentally with a ration containing trichloroethylene-extracted soybean oil meal. Gray et al. (1954) indicated the hematology of birds fed trichloroethylene-extracted soybean oil meal did not indicate the marked depression in numbers of blood cells found in birds severely affected with the hemorrhagic syndrome. Baker and Jacquette (1953), Gray et al. (1954), and Washko and Mushett (1955) reported hemorrhagic syndromes occurring in flocks that had not been fed trichloroethylene-extracted soybean oil meal.

All attempts to isolate or demonstrate an infectious organism from cases of hemorrhagic syndrome have been unsuccessful. Baker and Jaquette (1953) indicated that culture and embryo inoculation trials were negative. Cover et al. (1955) failed to transmit the syndrome by parenteral inoculation of chicks with blood, liver, spleen, and kidney from affected chickens. Embryo inoculations with tissues from affected birds were negative. Washko and Mushett (1955) reported that cultural examination of blood and tissues failed to yield an etiological agent. Hanley (1962) reported that aerobic and anaerobic culture attempts and bird inoculation trials were negative.

The significance of vitamin K in the role of hemorrhagic syndrome is not fully understood. Baker et al. (1953) reported that the addition of alfalfa leaf meal to the ration was of no benefit. Gray et al. (1954) stated that they could not relate some of their findings to vitamin K deficiency. Cover et al. (1955) made a detailed comparison of hemorrhagic syndrome and experimentally produced vitamin K deficiency. They noted that birds affected with hemorrhagic syndrome did not have the increased prothrombin time and massive hemorrhages found with vitamin K deficiency. They concluded that a comparison of the two syndromes showed them to be definitely distinct and dissimilar. Washko and Mushett (1955) found that prothrombin times and whole blood clotting times were within normal limits in most cases. Hence they did not consider it likely that vitamin K deficiency per se could be responsible for the hemorrhagic syndrome.

The role of toxic fungi in the hemorrhagic syndrome has been investigated by Forgacs and Carll (1955) and Forgacs et al. (1955, 1958, 1962). They stated that one of the paramount features of the hemorrhagic syndrome is the variability, both within and between affected flocks, in epizootiology, clinical symptoms, hematologic findings, course of mortality, and pathologic changes. They noted that these variations are strikingly similar to those observed among other hosts afflicted with known mycotoxicoses. Forgacs and Carll (1955) isolated various fungi from feed scattered in the litter of broiler houses where the hemorrhagic syndrome was enzootic. Some of these fungi, when cultured on a mixture of grains and subsequently dried, ground, and fed to one-day-old chicks, caused morbidity and mortality. At necropsy, hemorrhages were found in the subcutaneous tissue, skeletal muscles, heart, gastrointestinal tract, liver, and kidneys. Forgacs et al. (1958) reported attempts to produce the hemorrhagic syndrome in chickens under simulated field conditions. The chicks were placed on wood shavings and fed a broiler mash inoculated with a 0.5 per cent mixture of dry fungal substrate. The birds maintained on such litter manifested depres-

sion and diarrhea, and, on necropsy at the end of eight weeks, lesions similar to those of the hemorrhagic syndrome were found. The results of these studies indicate that the role of fungi in the hemorrhagic syndrome needs further investigation.

The widespread use of coccidiostats and the appearance of hemorrhagic syndrome occurred almost simultaneously. This observation prompted investigation into the role of coccidiostats in the hemorrhagic syndrome. Baker and Jaquette (1953), Cover et al. (1955), and Goldhaft and Wernicoff (1954) reported the occurrence of hemorrhagic syndrome in flocks that had not received sulfonamide medication. However, Gray et al. (1954) and Martheal and Velling (1961) indicated that in their cases of hemorrhagic syndrome the flocks had been treated with sulfaquinoxaline or other coccidiostats. In the author's examination of cases of hemorrhagic syndrome submitted to the diagnostic laboratory for the past ten years, most of the cases and particularly the severest cases have been associated with sulfonamide medication. That the condition is not a simple case of sulfonamide intoxication per se is indicated by the many flocks not showing hemorrhagic syndrome yet receiving the same amount of medication as affected birds.

Experiments have been conducted indicating that flocks can tolerate amounts of sulfonamides in excess of those used in feed as coccidiostats. Cuckler and Ott (1955) reported that the continuous administration of 0.05 per cent sulfaquinoxaline in the feed or of 0.025 per cent in water for as long as 12 weeks had no adverse effects on chickens. Gerry and Witter (1952) reported that histopathological examinations of tissues from 9-week-old birds fed 0.3 per cent sulfaquinoxaline in the mash for 3 weeks did not reveal any evidence of toxicity. However, the chickens fed this level of medication did not grow as well or make as efficient weight gains as the unmedicated controls. Sanger et al. (1956) reported that in their observa-

tions where certain drugs had been used, especially sulfonamides, the hemorrhagic syndrome suggested a manifestation of drug allergy

All cases of hemorrhagic syndrome have not been ascribed as being due to a single cause. It may be that similar clinical and pathological syndromes may be produced by different causes acting independently or simultaneously.

Control. In the light of our present knowledge, only certain precautionary measures can be suggested with no assurance that hemorrhagic syndrome will be prevented or cured. The use of coccidiostats, particularly sulfonamides, should be attended with caution in respect to dosage and duration of treatment. If there is gross evidence that mold is present in the feed hoppers or litter, corrective measures should be taken to eliminate this possible source of trouble. Although supplementary feeding of vitamin K in the form of alfalfa leaf meal or menadione bisulfate has been used in cases of hemorrhagic disease, there is no controlled evidence that this treatment has been successful.

Endocarditis. Vegetative endocarditis is rarely reported in the records of diagnostic laboratories. It probably would be found more frequently if the hearts of all birds necropsied were carefully opened. Kernkamp (1927) mentioned vegetative endocarditis as being associated with streptococcic peritonitis of chickens. Dauber and Katz (1943) reported vegetative endocarditis on the aortic and mitral valves associated with extensive pericarditis. The etiology of the pericarditis was undetermined and cultural attempts were not made. Most of the hearts affected with vegetative endocarditis were enlarged. On histological examination the vegetations were found to contain masses of bacteria and fibrin.

Povar and Brownstein (1947) described a large number of cases of valvular endocarditis observed during routine postmortem examination of birds dying from miscellaneous conditions on a large breed-

ing farm. Approximately 15 per cent of 551 females over 40 weeks of age had some degree of valvular endocarditis. Only 3 per cent of the birds between 10 weeks and 40 weeks of age were affected. It was noted that birds affected with chronic infections had a significantly greater incidence of valvular endocarditis than did birds dying of all other causes. Birds with salpingitis had the highest percentage of valvular endocarditis. Birds with hepatitis had the second highest percentage of heart lesions, and birds with lesions of bumblefoot ranked third in percentage of lesions.

They described the lesions as variable in size from small, punctiform, edematous nodules to yellowish, friable masses three-fourths of a centimeter in diameter. The smaller lesions lined the edge of the valves in a glistening row. The larger lesions were caseous and extremely friable. The semilunar valves of the pulmonary artery were most often affected followed by the right atrioventricular valves and the semilunar valves of the aorta in frequency. The left atrioventricular valves were affected with the lowest frequency but had the largest lesions. Cultures from the heart valve lesions yielded staphylococci and streptococci.

Gross and Domermuth (1962) reported the experimental production of endocarditis in chickens and turkeys by the intravenous inoculation of cultures of *Streptococcus faecalis*, *Staphylococcus aureus*, and *Pasteurella multocida* isolated from the livers of naturally infected birds. Bacteremia was followed by valvular lesions, and later infarcts were produced in the liver, spleen, and myocardium. The peak of mortality in birds with endocarditis occurred between the fifth and sixteenth day post inoculation.

DISEASES AFFECTING THE KIDNEYS

Cysts and agenesia. The kidneys occasionally have small cysts 5 mm. in diameter containing a clear amber-colored fluid distributed throughout the parenchyma. Rarely, a single large cyst may involve the kidney. It is not unusual to find agenesia

of one kidney in a healthy bird. The one remaining kidney apparently compensates for the added load and the bird is not unduly handicapped. The kidneys are frequently the site of tumors and inflammatory conditions associated with specific diseases. These conditions are discussed in their appropriate chapters in the text.

Avian nephrosis. Cosgrove (1962) reported his observations on a new syndrome in broilers occurring in the Delmarva Peninsula area. The condition has been termed "Gumboro disease" because of the prevalence of the disease in broilers in the vicinity of the town of Gumboro. The disease was first observed in 1957 and an increasing number of cases have been seen since that time.

Clinical signs. In a typical outbreak 10–20 per cent of the flock may be suddenly affected. An early sign is a watery, white diarrhea with soiling of the feathers in the vent region. This is followed by anorexia, depression, trembling, unsteady gait, prostration, and death. The mortality may range from 1 to 10 per cent. In the terminal stages, trembling of the neck and body is pronounced. The shanks show evidence of dehydration. Birds 2–15 weeks of age may be affected with most cases occurring at 5 weeks of age.

In a given group of birds the disease runs its course in 5–7 days. The disease spreads slowly from one pen to another. In some outbreaks the disease may affect only one pen. Outbreaks tend to reoccur on the same farm. The mortality ranges from 1 to 15 per cent with an average of 5 per cent.

Lesions. The most prominent and constant lesion is renal damage. In birds necropsied early in the course of the disease the kidneys may be nearly normal in color with only slightly visible tubules. In advanced stages of the disease the tubules and ureters are distended with urates and the kidneys are pale and enlarged. The degeneration and dysfunction of the kidneys is manifested by urate deposits on the serosal surfaces of the viscera in some birds. A catarrhal enteritis is present. There is

enlargement of the bursa of Fabricius which may have a white central core. The liver may have infarcts along the edges. Hemorrhages may be present in the leg and thigh muscles. The skeletal muscles show evidence of dehydration. Histopathological changes of the kidneys are cloudy swelling of the tubules. Winterfield and Hitchner (1962) indicated that both nephrosis and nephritis have been observed on microscopic examination. Blood studies indicate a low serum calcium and increased uric acid.

Etiology. Winterfield and Hitchner (1962) reported the isolation of two viruses which induced nephritis and nephrosis in chickens when inoculated in the eye. One virus was isolated in 1954 from the intestinal tract of a chicken with catarrhal enteritis. Other birds in the flock from which the original virus was isolated had intramuscular hemorrhages suggestive of hemorrhagic disease. The second virus was isolated from the kidneys of a 9-week-old bird which came from a flock with signs of nephrosis. This flock was vaccinated with infectious bronchitis vaccine at 1 week of age. When chicks were inoculated with either of the two virus isolates, mild respiratory symptoms were produced, mortality was low, and some birds had gross kidney lesions. By using serologic and cross-immunization studies, these isolates were determined to be related immunologically to known infectious bronchitis strains.

Gout. Although not of common occurrence gout has been observed in both young and mature turkeys and chickens (Jungherr, 1935; Schlotthauer and Bollman, 1931a). The predisposing factors are not always known but some cases have been associated with conditions that impose a stress on the kidneys such as a high protein diet, sodium bicarbonate intoxication, vitamin A deficiency, and bluecomb disease. The articular or visceral form may occur singly or in combination. Schlotthauer and Bollman (1931b) produced articular gout in turkeys by increasing the protein level of the feed to 40 per cent with the addition of horse meat and 5 per cent urea. Gout tophi appeared on the feet of those birds in which the blood values for uric acid were 15 mg. per cent for at least two weeks. In articular gout, urate deposits may occur on the articular surfaces and in the periarticular tissues (Fig. 39.19). Bullis and Van Roekel (1944) observed visceral gout in chicks less than 3–4 days of age. The losses were usually less than 5 per cent and the cause was unknown. In the visceral form, the kidney tubules are distended with urates and white deposits of uric acid crystals may be found on the surface of the viscera (Figs. 39.50 and 39.51). Lloyd *et al.* (1949) reported ex-

FIG. 39.49 — Gout in a mature hen. Urate deposits in toes, feet, joints, and tendon sheaths.

FIG. 39.50 — Visceral gout. Urate deposits in the kidneys of a chicken.

perimental production of articular gout involving the feet of young poults fed a 38 per cent protein ration (Fig. 39.52). The birds gradually recovered after the protein level was reduced to 20 per cent. The condition did not occur in groups fed a lower level of protein.

Snoeyenbos *et al.* (1962) reported naturally occurring gout in a flock of 400 eleven-month-old Broad Breasted White turkeys. About 20 per cent of the group were affected including both males and females. The flock was provided with adequate feed and water. The birds had been

eating a 20 per cent protein feed for 6 weeks. At necropsy, numerous tophi were observed in the periarticular tissues of the feet and uric acid crystals were present in the tendon sheaths and joints of the wings, hocks, and feet. Urate crystals and erosions were present on some of the articular surfaces. Serum uric acid levels were above 15 mg. per cent. Colchicine was fed to affected birds at a level of 0.4 mg. per pound of body weight per day for a week but this treatment failed to lower serum uric acid levels or alter the course of the disease. This dose was about 8 times the
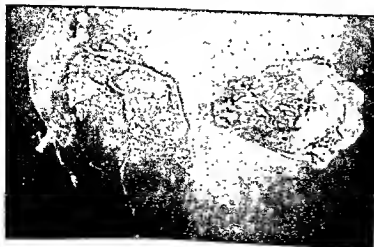
FIG. 39.51 — Visceral gout. Urate deposits on the surface of the liver and heart.

FIG. 39.52 — Swelling of the foot and toes in a turkey poult affected with gout.

maximum dose necessary to control the disease in man. The flock was placed on a 15 per cent protein ration for 11 days and then returned to the original ration. One month after the first signs were noted, the flock appeared to have recovered except for 6 severely affected birds. Egg production and hatchability were normal.

REPRODUCTIVE DISORDERS

Cystic right oviduct. In the developing female chick two ovaries and two oviducts are present. As the bird matures the left ovary and oviduct become functional and the right ovary and oviduct remain immature. However, there have been reports of finding two functional oviducts in fowl.

Of common occurrence is a cystic right oviduct. This structure may vary in size from a small one-inch elongated cyst (Fig. 39.53) to a ballooned sac containing a pint of clear watery fluid. The small cysts are of little consequence but large cysts compress the vital organs. Ascites, the accumulation of fluid in the abdominal cavity, is colloquially termed "water-belly" by poultrymen. In addition to a cystic oviduct, ascites may be produced by pathological conditions involving the heart, kidneys, liver, and mesentery.

Cystic left oviduct. Goldhaft (1956) reported the occurrence of massive cysts in the left oviduct and the dorsal ligament of the oviduct in a flock of White Leghorn
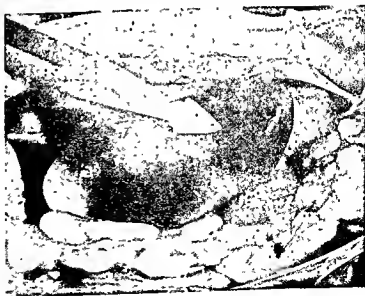
FIG. 39.53 — Cystic right oviduct in a chicken.

pullets. Where cysts occurred in the oviduct the anterior and posterior portions of the oviduct were normal but the middle of the oviduct was a blind sac distended with over a pint of clear fluid in some cases. It was estimated that 5 per cent of a flock of 2,800 seven-month-old birds were affected with these cysts during the previous 30-day period. The cause of the high percentage of cysts in this flock was not determined. Hutt et al. (1956) also observed discontinuous cysts in the oviduct similar to those described by Goldhaft (1956). They postulated that discontinuity of the oviduct probably resulted from accidental degeneration of part or parts of the Mullerian duct during the development of the embryo. They also described another defect in which the anterior end of the oviduct was closed by cohesion of the lips of the infundibulum causing a cystic oviduct.

Atresia of the oviduct. Finne and Vike (1951) described hereditary atresia of the oviduct in the region of the isthmus. The layers looked normal but the yolks were discharged into the body cavity. Peritonitis developed and the birds usually died at 5 to 6 months of age shortly after coming into production.

False layer. The term "false layer" has been used to describe the bird which looks like a normal layer but in reality does not lay eggs. Hutt et al. (1956) described the ovulating nonlayer hen as a bird that has the external characteristics of a layer, visits the nest regularly, yet does not lay eggs, as verified by trap nest records. This bird has a normal appearing ovary and oviduct but the infundibulum fails to engulf the ovum after it has been ovulated. These birds are excessively fat and have liquid or coagulated yolk in the body cavity.

Low production as a sequel to infectious bronchitis. Broadfoot et al. (1954) reported that natural infection of chicks with infectious bronchitis during the first week of age may interfere with later egg production. Flocks 7 months of age that had bronchitis as chicks were laying only 50 per cent whereas birds of the same breed-

ing that did not have bronchitis as chicks were laying at a normal rate. The birds in the poor laying flock looked healthy and had the appearance of good layers. Trapnesting records of the flock for 14 days showed that 43 per cent of the birds visited the nests on three to five successive days without laying. Some of these birds visited the nest as many as three times in a single day. Postmortem examination of 26 of these birds revealed that the viscera was surrounded by a heavy layer of mottled oily fat. Cheesy yolks with roughened and pitted surfaces were found in the body cavity. The oviducts were not full size and many were less than 20 per cent normal size. The striking fact about these birds was that the ovaries were found to be fully active. In a subsequent report, Broadfoot et al. (1956) experimentally infected chicks of different ages with virulent bronchitis virus and vaccine strains of bronchitis. The results of these experiments confirmed their field observations that exposure to virulent bronchitis virus during the first week of age produced an increasing number of false layers at maturity, and chicks exposed to a mild vaccine strain of bronchitis virus did not experience an adverse effect on egg production at maturity.

Impacted oviduct. Occasionally an impacted oviduct is observed in which the oviduct is occluded by masses of yolk, coagulated albumen, shell membranes, and, in some instances, fully formed eggs. Large masses of yolklike material may be found in the oviduct and upon transection these masses have the appearance of concentric rings.

Internal layer. In some birds soft-shelled eggs or fully formed eggs may be found in the peritoneal cavity. This indicates that the yolk progressed normally through the oviduct to a certain point and then reverse peristalsis discharged the egg into the body cavity. A bird with a large accumulation of eggs in the peritoneal cavity may assume a penguinlike posture.

Egg bound. "Egg bound" is the term used to describe the condition where an egg is lodged in the cloaca but cannot be

laid. It may result from inflammation of the oviduct, partial paralysis of the muscles of the oviduct, or the production of an egg so large that it is physically impossible for it to be laid. Young pullets laying an unusually large egg are more prone to an egg bound problem. This condition may be relieved by inserting a lubricated finger into the cloaca and exerting pressure on the abdomen with the other hand. If expulsion of the egg is not possible in this way then the egg may be held in position at the vent opening and the shell can be broken with a sharp object and the contents and pieces of the shell removed. If the chicken has been egg bound for a considerable period of time before the condition is detected there is apt to be an eversion of the cloacal tissues after the egg is removed. The blood-stained tissues invite cannibalism and the bird should be placed in separate quarters until fully recovered.

Ovarian disorders. The ovary reflects the general health of the mature bird. Many infectious diseases and physiological disturbances immediately produce retrogressive changes in the ovary. The normal, yellow, turgid ovum may become wrinkled and the contents almost black with hemorrhage. At other times the yolk is coagulated or "cooked" in appearance. Discolored, pedunculated, and inspissated ova are the hallmark of pullorum disease. Cauliflowerlike growths from the ovary are indicative of lymphomatosis. Multiple cystic ova filled with clear fluid may occur occasionally.

Abnormal eggs and depressed production. Eggs differing in size and shape may be laid in any flock but in the average healthy flock there is remarkable uniformity of the eggs. There are a number of conditions which affect shell quality, internal quality, and rate of lay. Wilson (1949) found that environmental temperatures had a marked effect on the strength and thickness of the shell. As the temperature rises above 70° F., the blood calcium drops progressively; at 100° F. the shell averages about two-thirds the normal thick-

ness. This would explain why the eggshell is thicker and stronger in the winter months. Increased humidity also has an unfavorable effect on shell thickness but not as marked as increased temperature. Jull (1930) indicated that the production of soft-shelled eggs is most prevalent in the spring when production is at its peak. Respiratory diseases such as infectious bronchitis and Newcastle disease may affect the size, shape, shell texture, and internal quality of eggs (Gordeuk and Bressler, 1950; Biswall and Morrill, 1954).

Mann and Keilin (1940) reported sulfanilamide to be a very powerful inhibitor of the enzyme carbonic anhydrase. Since then several reports have appeared in the literature describing the inhibitory effects of certain sulfonamides on the formation of eggshells. Hinshaw and McNeil (1943) found that a single dose of sulfanilamide, 0.5 grain per pound of live weight, caused turkeys to lay eggs with very thin shells or eggs without shells the next day. When they fed 1.5 grains of sulfanilamide per pound of live weight to leghorn hens, the same result was produced. This inhibitory action of small dosages of sulfanilamide on the secretion of shell material was confirmed by Benesch et al. (1944), Gutowska and Mitchell (1945), and Tyler (1950). Scott et al. (1944) observed that in addition to thin shells, sulfanilamide also caused a bleaching of the pigment in brown eggs. Bankowski (1948) reported a decrease in egg production following sulfamerazine and sulfamethazine medication. Mehring et al. (1955) reported that two unsubstituted sulfonamides, Diamox and benzenesulfonamide, caused pullets to lay eggs with very thin shells or with no shells beginning with the first egg laid after the initial dose. Nicarbazin, a coccidiostat, may cause mottled yolks, loss of shell pigment, decreased egg size, and low egg production (Weiss, 1957; Baker et al., 1957). Arasan, a fungicide used in the treatment of seed corn, has been reported to cause misshapen and soft-shelled eggs, retarded production, and finally complete cessation of production (Waible et al., 1955; Johnson et al., 1955).

Trematodes and ascarids sometimes enter the cloaca and pass up the oviduct where they are swept along with the yolk and albumen and become encased by the shell.

Cloacitis. Cloacitis, or vent gleet as it is commonly known, is a chronic inflammatory process of the cloaca with a very offensive odor. A yellow diphtheritic membrane may form on the mucosal surface of the vent and urates and inflammatory exudate contaminate the skin and feathers beneath the vent. The disease is more common in laying hens than in males. A very small percentage of birds in a flock are affected at one time and the disease does not appear very contagious. The specific cause of this disease is unknown. Transmission experiments carried out by Gwatkin (1925) and Scherago (1925) were unsuccessful. Removal of affected birds is advised in commercial flocks. Valuable birds can be treated by cleansing the affected area and applying a broad-spectrum antibiotic in dust or ointment form.

Cage layer fatigue. The marked increase in the number of birds kept in cages during the past decade has given rise to a new problem. The condition, called cage layer fatigue, is characterized by inability of the birds to stand and marked fragility of the bones (Fig. 39.54). The bones are not soft but are exceedingly thin and break or splinter at the slightest pressure. In addition to paralysis, the rearing of birds in cages has created a marketing problem. The marked fragility of the bones results in fractures when the birds are removed from the cages and more injury occurs when the birds are subjected to the pounding of the picking machines in the dressing plants. The bone splinters become lodged in the meat, creating processing problems.

It has been observed that birds will recover in 4-7 days if removed from the cages and placed on the floor (Couch, 1955). Francis (1957) noted significant differences in the incidence of cage layer fatigue between various strains. During a 10-week observation period 0.65 per cent of one strain was affected compared to a high of 3.95 per cent in another strain. Eighty per cent of the birds recovered when removed from the cages. Couch (1957) stated that bone ash, blood calcium, and blood phosphorus values on selected early cases were all within the normal range which would differentiate the condition from rickets. According to Grumbles (1959) this condition occurs more often during the summer months in young pullets that are producing at a high rate and have a good feed efficiency ratio. The percentage af-

FIG. 39.54 — White Leghorn affected with cage layer fatigue showing characteristic squatting position.

fected will vary from 1 to 20 per cent. Fisher, quoted by Grumbles (1959), stated that in his experience, cage fatigue has not occurred when the calcium level of the diet was maintained at 2.5 per cent.

CONGENITAL AND INHERITED CONDITIONS

Congenital loco. Durant (1926, 1927) first noted this nervous disorder in newly hatched White Leghorn chicks. Subsequently, Knowlton (1929) conducted breeding trials and concluded that the defect was inherited as a simple Mendelian recessive. An apparently identical syndrome was reported in turkey poults by Cole (1957). After a thorough investigation of the genetic factors involved he concluded that the condition was an obligate postnatal lethal syndrome caused by the homozygous state of the recessive gene *lo*. Hatchability is not affected and the poults are in excellent physical condition. Symptoms manifested are opisthotonus, or sagging of the head and neck until the beak touches the floor. The birds can stand normally for only a few seconds at a time and then in-

voluntarily or in response to external stimuli they thrust themselves vigorously into a backward somersault. They lie on their backs or sides kicking aimlessly (Fig. 39.55). The anatomical defect responsible for this syndrome is unknown. The brain appears normal upon gross and microscopic examination. Affected birds die since they are unable to eat or drink.

Congenital alopecia. Congenital alopecia is occasionally encountered in newly hatched chicks. Certain avian hybrids develop a partial alopecia which is characteristic of the cross breeding.

Congenital opisthotonus. Caskey *et al.* (1944) described congenital opisthotonus occurring in chicks at hatching due to manganese deficiency in the maternal diet. The symptoms persisted at maturity even though adequate manganese was given. The progeny resulting from the mating of affected males and females did not manifest opisthotonus thus indicating it was not an inherited characteristic.

Cerebellar hypoplasia. Avian cerebellar hypoplasia and degeneration according to Winterfield (1953) is probably an inherited

FIG. 39.55 — Congenital loco in turkey poults. Affected birds manifest opisthotonus and thrust themselves over backward.

defect. The condition was first observed in 12-week-old pullets in 15 different flocks with 3 to 10 per cent becoming affected. Affected birds did not die and came into production at 6 months of age. Symptoms were weaving and bobbing of the head, and when the birds were excited they became ataxic. Gross pathology was confined to the cerebellum which was about one-fourth normal size. Neuronal degeneration was present in the cerebellar cortex and there was degeneration and disappearance of the Purkinje cells. In England, Markson et al. (1959) described a similar condition affecting Light Sussex pullets.

Crooked toes. Crooked toes may occur in young or adult birds. This condition should not be confused with "curly toe paralysis" which is caused by riboflavin deficiency. In the crooked toe condition the toes have a lateral curvature but the bird still walks on the plantar surface of the foot (Fig. 39.56). However, in "curly toe paralysis" the toes curl under and the bird walks on the dorsal surface of the toes. Birds with riboflavin deficiency have difficulty in walking and rest on their hocks when not trying to walk. In "curly toe paralysis" a histological section of the sciatic nerve will enable a positive diagnosis to be made.

The tendency for crooked toes is inherited and any birds so affected should not be used for breeding. Black et al. (1952) reported strain differences in the manifestation of crooked toe condition in chicks when brooded under the bright emitter type of infra-red lamp. Chicks from one strain manifested none of the abnormality whereas 100 per cent of the offspring from crooked-toed parents of another strain had crooked toes when brooded under infrared lamps. However, when the two stocks were reared under ordinary electrical heating, crooked toes did not develop in either strain. This is an example of the interactions which frequently occur between inheritance and environment. Rearing chicks on a smooth surface such as a newspaper will increase the number of cases of crooked toes if the birds carry this genetic defect. Crooked toes are under the influence of genetic factors; however, these genes express themselves differently in various environments.

Scoliosis. This condition commonly spoken of as wry neck is characterized by torsion and lateral curvature of the neck (Figs. 39.57 and 39.58). It is most commonly observed in birds of growing age

FIG. 39.56 — Crooked toe condition in a three-week-old chick.

FIG. 39.57 — Wry-neck condition in a young chicken.

FIG. 39.58 – Wry neck. Same chicken as Fig. 39.57 with neck exposed revealing torsion and lateral deviation.

and usually only an occasional bird in a flock is affected. Although birds with this affliction manage to eat and drink without too much difficulty it is generally best to remove them from the flock.

Coloboma of the iris. This condition is manifest as a unilateral or bilateral teardrop shape of the pupil or as a circular pupil with a defect in the iris. Wilcox (1958) presented evidence that the condition was inherited in White Leghorns by finding coloboma in 22 per cent of the offspring obtained from mating affected birds. Because of the dark pigmentation of the iris in chicks the defect was not readily discernible until six weeks of age. The defect was much more obvious when observed in bright light in which the iris was maximally constricted. In some of the offspring without coloboma, a pronounced bulging of the cornea occurred presumably from increased intraocular pressure in the anterior chamber of the eye. In one case bulging developed in an eye previously classified as coloboma. Another abnormality observed occasionally in this stock was a sunken eye, which was reduced considerably in size. Recognition of the existence of coloboma in chickens is important in making a differential diagnosis of ocular lymphomatosis. A histological examination of the eye would enable one to differentiate between these two conditions.

REFERENCES

Angstrom, C. I.: 1961. Personal communication.
———: 1962. Personal communication.
Adersen, J.: 1918. On the occurrence of the so-called round heart disease in Denmark. Proc. 8th World's Poultry Congress 702.
Baker, H. R., and Jaquette, D. S.: 1953. Observations concerning the "hemorrhagic syndrome" in poultry. Proc. 25th Ann. Conf. of Lab. Workers in Pullorum Disease Control. Amherst, Mass.
Baker, R. C., Hill, F. W., Van Tienhoven, A., and Bruckner, J. H.: 1957. The effect of Nicarbazin on egg production and egg quality. Poultry Sci. 36:718.
Bankier, J. C.: 1954. Poultry disease problems in British Columbia. Proc. Am. Vet. Med. Assn. 91st Ann. Mtg. 350.

Bankowski, R. A.: 1948. A decrease in egg production following sulfamerazine and sulfamethazine medication. Jour. Am. Vet. Med. Assn. 113:49.

Barber, C. W.: 1947. Studies on the avian leukosis complex. Cornell Vet. 37:349.

Bass, C. C.: 1939. Control of "nose-picking" form of cannibalism in young closely confined quail. Proc. Soc. Exper. Biol. and Med. 40:488.

Benesch, R., Barron, N. S., and Mawson, C. A.: 1944. Carbonic anhydrase, sulfonamides, and shell formation in the domestic fowl. Nature 153.138.

Biswall, G., and Morrill, C.: 1954. The pathology of the reproductive tract of laying pullets affected with Newcastle disease. Poultry Sci. 33.880

Black, D. J. G., Getty, J., and Morris, T. R.: 1952. Infra-red brooding and the crooked toe problem in chicks. Nature 170:167.

Blaxland, J. D., and Markson, L. M.: 1947. Toxic heart degeneration, or "round heart disease" of poultry. Brit. Vet. Jour. 103:401.

Bornstein, S., and Samberg, Y.: 1954. Field cases of vitamin K deficiency in Israel. Poultry Sci. 33:831.

Bragg, D. D.: 1953. An attempt to determine the cause of curled or deformed tongues in young Beltsville White Turkeys. Poultry Sci. 32:294

Brion, A. J., and Fontaine, M.: 1958. A hemorrhagic and rachitic-like syndrome in chickens due to nitrofural medicated feed. Poultry Sci. 37:1071.

Broadfoot, D. I., Pomeroy, B. S., and Smith, W. M.: 1954. Effects of infectious bronchitis on egg production. Jour. Am. Vet. Assn. 124:128.

———. Pomeroy, B. S., and Smith, W. M.: 1956. Effects of infectious bronchitis in baby chicks. Poultry Sci. 35:757.

Bullis, K. L., Snoeyenbos, G. H., and Van Roekel, H.: 1950. A keratoconjunctivitis in chickens. Poultry Sci. 29:386.

———, and Van Roekel, H.: 1944. Uncommon pathological conditions in chickens and turkeys. Cornell Vet. 34:312.

Caroaghan, R. B. A.: 1958. Rupture of the gastrocnemius tendon in fowls. Brit. Vet. Jour. 114.1.

Cartrite, H. T.: 1951. Hemorrhagic disease. Abstract of paper presented at poultry pathologists conference. Bear Mt. Inn, Stony Point, New York.

Caskey, C. D., North, L. C. and Heuser, G. F.: 1944. A chronic congenital ataxia in chicks due to manganese deficiency in the maternal diet. Poultry Sci. 23:516.

Chute, H. L.: 1950a. Rupture of the gastrocnemius tendon in chickens. Canad. Jour. Comp. Med. 14.218.

———: 1950b. Ruptured gastrocnemius tendons. Proc. 22nd Ann. Conf. of Lab. Workers in Pullorum Disease Control. Univ. of Vermont, Burlington, Vermont.

Cole, R. R.: 1957. Congenital loco in turkeys. Jour. of Heredity 48:173.

Corner, A. H., Isa, J. M., and Bannister, G. L.: 1959. Xanthomatosis in White Leghorns in Canada. Canad. Jour. Comp. Med. 23.199.

Cosgrove, A. S.: 1962. An apparently new disease of chickens—avian nephrosis. Avian Dis. 6:385

Couch, J. R.: 1935 Cage layer fatigue Feed Age 5 55.

———: 1957. Caged fatigue. Everybody's Poultry Magazine (March) 12.

Cover, M. S., Mellen, W. J., and Gill, E.: 1955. Studies of hemorrhagic syndromes in chickens. Cornell Vet. 45:366.

Creek, R. D., and Dendy, M. Y.: 1957. The relationship of cannibalism and methionine. Poultry Sci 36:1093.

Cuckler, A. C., and Ott, W. H.: 1955. Tolerance studies on sulfaquinoxaline in poultry. Poultry Sci. 34:867.

Dauber, D. V., and Katz, L. N.: 1943. Experimental atherosclerosis in the chick. Arch. of Path. 36:473.

Devos, A.: 1962. Xanthomatose bij graspurkieten. Vlaams Diergeneeskundig tijdschrift. 31:211.

Dickinson, E. M., and Clark, W. G.: 1946. Brooder stove residue burns on turkey poults Cornell Vet. 36:514.

Durant, A. J.: 1926. Inherited incoordination of muscles in newly hatched chicks. Mo. Agr Exper. Sta. Bul. No 241:60.

———: 1927. Inherited incoordination of muscles in newly hatched chicks. Mo. Agr. Exper. Sta. Bul. No. 256:102.

Eber, A., and Pallaski-Eber, R.: 1934. Die durch obduktion festzstellbaren Geflügelkrankheiten S. 251 Verlag M u H. Schaper, Hannover.

Evelcth, D. F., and Goldaby, A. I.: 1953. Toxicosis of chickens caused by trichloroethylene-extracted soybean meal. Jour. Am. Vet. Med. Assn. 123.38.

Faddoul, G. P., and Ringrose, R. C.: 1950. Avian keratoconjunctivitis. Vet. Med. 45:492.

Finne, I., and Vike, N.: 1951. A new sub-lethal factor in hens. Poultry Sci. 30:455.

Fischel, W. G.: 1946. Enzootic fatal syncope (toxic heart degeneration) of fowls. Australian Vet. Jour. 22.144

Kernkamp, H. C. H.: 1927. Idiopathic streptococcic peritonitis in poultry. Jour. Am. Vet. Res. 23:585.

Knowlton, F. L.: 1929. Congenital loco in chicks. Oregon Agr. Exper. Sta. Bul. No. 253:1.

Kona, E., and Belobrad, G.: 1958. Beitrag zum Vorkommen des enzootischen Herztodes bei Huhnern. Veterinärmedezin 11:324.

Kull, R. E.: 1948. Prevention and treatment of cannibalism and feather eating in fowls. Official Rep. 8th World's Poultry Cong. 1:124.

Kurtze, H.: 1948. Ein Fall von plotzlichem Herztod bei einer Ente. Deutsch. tierärztl. Wochenschr. 55:201.

Levine, P. P.: 1952. Ascites and nephritis of traumatic origin in day old chicks. Proc. 24th Ann. Conf. Lab. Workers in Pullorum Disease Control. Univ. of Maine, Orono, Maine.

———: 1958. Case report — round heart disease in the United States. Avian Dis. 2.530.

Lloyd, M. D., Reed, C. A., and Fritz, J. C.: 1949. Experiences with high protein diets for chicks and poults. Poultry Sci. 28.69.

Lonsdale, D. B., Vondell, R. M., and Ringrose, R. C.: 1957. Debeaking at one day of age and the feeding of pellets to broiler chickens. Poultry Sci. 36:565.

Luke, D.: 1947. Round heart disease in poultry. Vet. Jour. 103:17.

Mann, T., and Keilin, D.: 1940. Sulfanilamide as a specific inhibitor of carbonic anhydrase. Nature 146:164.

Markson, L. M., Carnaghan, R. B. A., and Young, G. B.: 1959. Familial cerebellar degeneration and atrophy — a sex-linked disease affecting Light Sussex pullets. Jour. Comp. Path. and Therap. 69.225.

Marthedal, H. E., and Velling, G.: 1961. Hemorrhagic syndrome in poultry Brit. Vet. Jour. 117:557.

Mathey, W. J.: 1956. A diphtheroid stomatitis of chickens apparently due to *Spirillum pulli*, species nova. Am. Jour. Vet. Res. 17:742.

———, and Zander, D. V.: 1955. Spirochetes and cecal nodules in poultry. Jour, Am. Vet. Med. Assn. 126:478.

Matuke, M.: 1942. Ein beitrag zum sogenannten "Eierherzen" bei Huhnern. Berl. Münch. tierärztl. Wochenschr. 283.

Mehring, A. L., Jr., Titus, H. W., and Brumbaugh, J. H.: 1955. Effects of two sulfonamides on the formation of egg shells. Poultry Sci. 34:1385.

Meinecke, C. F., Flowers, A. I., and Beasley, J. N.: 1962. Observations of xanthomatosis in chickens. Poultry Sci. 41:1207.

Miller, W. M., and Bearse, G. E.: 1937. The cannibalism-preventing properties of oats. Poultry Sci. 16:314.

Morgan, W.: 1957. Effect of day-old debeaking on the performance of pullets. Poultry Sci. 36.208

Neal, W. M.: 1956. Cannibalism, pick-outs and methionine. Poultry Sci. 35:10.

Nelson, C. L.: 1952. Cannibalism. Iowa Vet. 23 28.

Newberne, P. M., Muhrer, M E., Craghead, R., and O'Dell, B. L.: 1956. An abnormality of the proventriculus of the chick. Jour. Am. Vet. Med. Assn. 128 555.

Newsom, I. E., and Feldman, W. H.: 1920. Sod disease of chickens (Vesicular dermatitis). Colo. Agr. Exper. Sta. Bul. 262.

Ostrander, C. E.: 1957. Control cannibalism in your poultry flock. Cornell Ext. Bul. 992.

Peckham, M. C.: 1955. Xanthomatosis in chickens. Am. Jour. Vet. Res. 16.580.

Peek, M.: 1958. Ergot and ergot-like fungi as the cause of vesicular dermatitis (sod disease) in chickens. Jour. Am Vet. Med. Assn. 132:529.

Povar, M. L., and Brownstein, B.: 1947. Valvular endocarditis in the fowl. Cornell Vet. 37:49.

Pritchard, W. R., Rehfeld, C. E., and Sautter, J. H.: 1952. Aplastic anemia of cattle associated with ingestion of trichloroethylene extracted soybean oil meal. Jour. Am. Vet. Med. Assn. 121:1.

Robertson, E. I., Angstrom, C. I., Clark, H. C., and Shinnn, M.: 1949. Field research on "stunted chick" disease. Poultry Sci. 28:14.

Sadek, S. E., Hanson, L. E., and Alberts, J. O.: 1955 Suspected drug-induced anemias in the chicken. Jour. Am. Vet. Med. Assn. 127:201.

Sanger, V. L., Chamberlain, D M., Cole, C. R., Docton, F. L., and Farrell, R. L.: 1953. A disease of turkeys characterized by deformity of the tongue. Jour. Am. Vet. Med. Assn. 122:207.

———, Yacowitz, H., and Moore, E. H.: 1956. Micropathological changes in an experimental hemorrhagic syndrome in chickens fed sulfaquinoxaline and suggested cause of the disease. Am. Jour. Vet. Res. 17:766.

Sassenhoff, I.: 1947. Enzootischen Herztod bei Hühnern. Tierärztl. Umshau. 15/16:181.

Saunders, C. N.: 1958. Kerato-conjunctivitis in broiler birds. Vet. Rec. 70:117.

Sautter, J. H., Rehfeld, C. E., and Pritchard, W. R.: 1952. Aplastic anemia of cattle associated with ingestion of trichloroethylene-extracted soybean oil meal. II. Necropsy findings in field cases. Jour. Am. Vet. Med. Assn. 121:73.

Scherago, M.: 1925 Ulcerative cloacitis in chickens. Jour. Am. Vet. Med. Assn. 67:232.

Schlotthauer, C. F., and Bollman, J. L.: 1934a. Spontaneous gout in turkeys. Jour. Am. Vet. Med. Assn. 85:98.

———, and Bollman, J. L.: 1934b. Experimental gout in turkeys. Proc. Staff Meet. Mayo Clinic 9:560.

Schröter, A.: 1952. Über den enzootischen Herztod bei Hühnern. Monatsheft. Vet. Med. 7:271.

Scott, H. M., Jungherr, E. L., and Matterson, L. D: 1944. The effect of feeding sulfanilamide to the laying fowl. Poultry Sci. 23:446.

Shelton, D. C., Anderson, G. C., Bletner, J. K., Weakley, C. E., Jr., Cook, R. C., and Lewis, W. R.: 1954. The role of coccidiostats and growth stimulators in the chick hemorrhagic condition. Poultry Sci. 33:1080.

Siegmann, O., and Woernle, H.: 1954. Beitrag zum enzootischen Herztod der Hühner. Arch. f. Exper. Vet. Med. 8:465.

Snoeyenbos, G. H., Reynolds, J. M., and Tzianabos, T.: 1962. Articular gout in turkeys. Avian Dis. 6:32.

Stafseth, H. J.: 1934. Diseases of adult poultry. Mich. State Coll., Ext. Bul. 54 (Revised).

Thoonen, J., Hoorens, J., and Van Meirhaeghe, E.: 1959. Xanthomatose beim Huhn. Arch. f. Geflugelkunde 23:314.

Trenchi, H.: 1960. Ingestion of *Ammi visnaga* seeds and photosensitization—the cause of vesicular dermatitis in fowls. Avian Dis. 4:275.

———: 1962. A comparative study of the lesions produced by ergot and photosensitization induced by *Ammi visnaga*. Fourth Pan Am. Cong. of Vet. Med. and Zootechnics. Mexico City, Mexico.

Tyler, C.: 1950. The effect of sulphanilamide on the metabolism of calcium, carbonate, phosphorus chloride, and nitrogen in the laying hen. Brit. Jour. of Nutrition 4:112.

Van Ness, G.: 1946. *Staphylococcus citreus* in the fowl. Poultry Sci. 25.647.

Waibel, P. E., Pomeroy, B. S., and Johnson, E. L.: 1955. Effect of Arasan-treated corn on laying hens. Science 121:401.

Wannop, C. C.: 1957. Some observations on the hemorrhagic syndrome in chicks. World Poultry Sci. Jour. 13:310.

Washko, F. V., and Mushett, C. W.: 1955. Some observations on the pathology of the hemorrhagic condition of chickens. Proc. Am. Vet. Med. Assn. 360.

Weaver, C. H., and Bird, S.: 1934. The nature of cannibalism occurring among adult domestic fowls. Jour. Am. Vet. Med. Assn. 85:623.

West, J. E.: 1957. Further comments on the effect of nicarbazin on the egg. Poultry Sci. 36:589.

Wilson, F. H.: 1958. Studies on the inheritance of coloboma of the iris in the domestic fowl. Jour. of Heredity 49:107.

Willimon, C. P., and Morgan, C. L.: 1953. The effect of minor nutrient mineral elements in the diet of chickens on feather pulling and cannibalism. Poultry Sci. 32:309.

Wilson, J. E.: 1948. Round heart disease in poultry in Scotland. Proc. 8th World's Poultry Congress, 697.

———: 1957. Round heart disease in poultry. Jour. Comp. Path. and Therap. 67:239.

———, and Siller, W. G.: 1954. Round heart disease in the fowl. Jour. Comp. Path. 64:41.

Wilson, W. O.: 1949. High environmental temperatures as effecting the reaction of laying hens to iodized casein. Poultry Sci. 28:581.

Winterfield, R. W.: 1953. Avian cerebellar hypoplasia and degeneration. Jour. Am. Vet. Med. Assn. 123:136.

———, and Hitchner, S. B.: 1962. Etiology of an infectious nephritis-nephrosis syndrome of chickens. Am. Jour. Vet. Res. 23:1273.

Wright, G. W., and Frank, J. F.: 1957. Ocular lesions in chickens caused by ammonia fumes. Canad. Jour. Comp. Med. 21:225.

Wright, M. M., and Temperton, H.: 1955. Curled tongue in turkey poults. Vet. Rec. 67.510.

Yacowitz, H., Ross, E., Sanger, V. L., Moore, E. H., and Carter, R. D.: 1955. Hemorrhagic syndrome in chicks fed normal rations supplemented with sulfaquinoxaline. Proc. Soc. Exper. Biol. and Med. 89:1.

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40

Poisons and Toxins

The literature contains many reports of acute and chronic poisoning of birds due to the ingestion of toxic substances, but the losses from these conditions are insignificant compared with the losses experienced from various other diseases. The majority of cases of poisoning are accidental or due to a poor system of management. When birds are confined to small units or the supply of natural food on the range is limited due to unfavorable weather conditions, they may consume any succulent food available regardless of its palatability or toxicity.

Losses in birds may be attributed to autointoxication, bacterial intoxication, and poisoning by drugs and chemicals, as well as by various phytotoxins, insects, and food constituents. Some of these agents are comparatively rare, and little is known

about them, but the more common ones have been investigated, and the toxic as well as the lethal dosages have been determined. In cases of poisoning in birds, positive diagnoses in most instances are made too late for effective treatment, but if the cause is definitely established, it may be removed and further losses avoided.

The body defenses which act to destroy the toxic action of poisons vary considerably in different animals. There is also a variation in the tolerance of certain species for toxic agents. As the result of extensive investigations, Sherwin and Crowdle (1922) found that the action in the bodies of fowls was similar to that of other animals regarding the detoxication of various poisonous substances.

AUTOINTOXICATION

Autointoxication may be defined as self-poisoning due to the absorption of the waste products of metabolism or of the products of decomposition within the in-

testine. In young chicks which are raised under artificial conditions, autointoxication is more frequently experienced as the result of injudicious feeding practices. The feeding of bulky foods rich in crude fiber which may occlude the digestive tract may prevent proper elimination and cause the absorption of decomposed contents of the intestines. Chicks which are raised in confinement and are supplied with chopped green feed often consume quantities of coarse fibrous stems which obstruct the intestinal tract. This condition occurs most frequently in young poults which are raised in confinement and supplied with green feed containing short pieces of fibrous stems. Large numbers of poults may be lost in a short time following such feeding procedures. The use of hay chaff for litter in poultry houses frequently results in the ingestion of indigestible fibrous material which may cause obstruction of the intestinal tract.

The symptoms observed in birds suffering from autointoxication include loss of appetite, increased water consumption, and depression, followed by weakness and prostration. Nervous symptoms typical of a generalized toxemia may appear shortly before death.

Occasionally sudden death occurs among apparently healthy turkeys and is caused by the consumption of large numbers of grasshoppers without any appreciable amount of other food being taken at the same time. Death is caused by the hard parts of the grasshopper, particularly the spined legs which irritate the mucosa of the digestive tract and frequently puncture the walls of the crop and intestines. Nongallinaceous birds are apparently not affected in this manner.

BACTERIAL TOXINS

Although the losses in birds attributed to bacterial toxins are not considered to be of great economic importance, they occasionally result in heavy losses in individual flocks. The only organism of this type which is important in the consideration of poultry diseases is *Clostridium*

botulinum. No significant lesions are found in botulism, and a positive diagnosis is based upon demonstration of the organism and its toxin.

MOLDS AND FUNGI

Molds and fungi frequently attack grains and forage crops in the field and in storage when conditions are favorable for the development of these organisms. They frequently produce toxins which are poisonous to mammals and birds, and in some instances have caused considerable losses. As a rule, birds are less susceptible to poisoning by molds or fungi than are the common species of domesticated animals. Moldy grains have long been considered as dangerous for stock feed, but they are invariably fed until losses occur. The appearance of the grain is no index as to its toxicity. Some of the worst-looking grains may prove to be nontoxic, while brighter and better-appearing grains may be extremely poisonous. Chickens have been fed moldy corn infected with species of Diplodia, Aspergillus, Mucor or Rhizopus, Penicillium, and various bacterial organisms without any unfavorable results. Scabby barley heavily infested with *Gibberella saubinetii* has been fed to fowls without apparently affecting their health or egg production. Wheat damaged by the so called stinking smut was used quite successfully as a poultry feed, although it is generally admitted that the feeding value of such grain is impaired by the action of the smut.

Even though the practice of feeding salvage grains as well as those infected with molds and fungi is quite common, extreme care should be taken in the selection of the grain constituents, and only feed of good quality should be used as poultry feed.

Brazilian groundnut poisoning (*Aspergillus flavus* toxin). In England during 1960, Blount (1961) reported that at least 500 cases of turkey "X" disease were diagnosed and indicated it was estimated that more than 100,000 turkeys died. Intensive investigations by various British workers,

Sargeant and O'Kelley (1961) soon made it apparent that this was a new disease. A feeding trial reported by Blount (1961) produced 100 per cent mortality in 25 poults between 2 to 3 weeks of age and this was the first evidence that proprietary feeds were causing the losses. Subsequent investigation revealed that Brazilian groundnut meal was the one ingredient present in all the toxic feeds. It was discovered that the toxic principle was aflatoxin, a toxic metabolite produced by strains of Aspergillus flavus contaminating the peanut meal (Sargeant et al., 1961). Samples of groundnut meal from Africa and India also found to be toxic by Carnaghan and Sargeant (1961). These investigations led to the discovery that strains of Aspergillus flavus are among the commonest fungal contaminants of cereal grains and the possibility exists that these grains could be a source of toxin. The first report of Brazilian nut poisoning in North America was by Archibald et al. (1962) who reported the disease in Canadian chickens.

Signs and lesions. Turkeys are generally affected at 4 to 6 weeks of age but losses may occur up to 16 weeks of age. Death usually follows within a week after symptoms are noticed. Affected birds will eat litter and there is a gradual loss of appetite. The poults are lethargic, the wings droop, and the feathers are ruffled and broken. Occasionally, nervous symptoms are present and the birds may die in a state of opisthotonus with the legs extended backwards. Mortality is variable but usually is high with 50 to 90 per cent loss not uncommon (Blount, 1961). Chickens fed diets similar to those which kill turkeys, pheasants, and ducklings have retarded growth but low mortality.

The primary lesions in poults are enteritis and nephritis. The liver may have congestion, petechial hemorrhages, or pale necrotic areas. Blount (1961) reported that the blood may be watery and fail to clot normally.

Ducklings are more susceptible to afla-

toxin than turkeys, pheasants, or chickens and this sensitivity makes them the bird of choice when testing feed for toxicity. Clinical signs are manifested by diminished feed intake and poor growth. A conspicuous change sometimes observed in young white-skinned ducklings is lameness and a purple discoloration of the feet and legs caused by subcutaneous hemorrhage. Young ducklings develop ataxia and convulsions shortly before death and manifest opisthotonus. It should be noted that opisthotonus is characteristic of the terminal stage in duck virus hepatitis.

Internal lesions vary according to survival time and age of the birds (Asplin and Carnaghan, 1961). Ducklings dying at one week of age have slightly enlarged puttycolored livers and pale, swollen kidneys. Petechia are present in the kidneys and pancreas. Older ducklings have a pale reticulated network throughout the liver and this is accompanied by atrophy and cirrhosis. Nodular hyperplasia of the liver is found in chronic cases. Ascites and hydropericardium may be present along with a subcutaneous transudate.

Histopathology. The most prominent histopathological changes in turkeys as reported by Wannop (1961) were degeneration of the liver cells and bile duct hyperplasia. Similar hepatic changes were observed in ducklings (Asplin and Carnaghan 1961). The pancreas had diffuse areas of acinar degeneration. The kidneys had congestion of the tubular sinusoids and degeneration of the proximal convoluted tubules. In 3- to 4-month-old chickens, lymphoid hyperplasia occurred particularly in those areas where parenchymal cell regeneration was seen. By 4 months of age multiple circumscribed areas of lymphoid hyperplasia were found scattered throughout the liver (Asplin and Carnaghan, 1961). Pancreatic and renal changes were similar to those in ducklings.

A positive diagnosis of this disease would necessitate the demonstration of aflatoxin in the feed. At present, means of testing for this toxin are beyond the scope of the

average diagnostic laboratory. Biological testing could be performed by feeding the suspect feed to ducklings.

Ergot. Ergot poisoning is occasionally responsible for extensive losses in poultry. Ergot is the sclerotium of the fungus *Claviceps purpurea* which infects the seed-bearing heads of maturing rye and other grains (Fig. 40.1). In European countries where rye is commonly used as poultry feed, ergotism is frequently encountered. Ergot is distasteful to chickens and if other feed is available they will not eat it. In acute poisoning the comb becomes wilted and cyanotic. The birds become depressed and do not eat but have abnormal thirst accompanied by diarrhea. General debilitation is followed by convulsions, paralysis, and death. In chronic cases the comb, wattles, and toes may become discolored and necrotic and may slough. Internal lesions consist of enteritis in conjunction with degenerative changes in the heart, liver, and kidneys. Chickens will make a prompt recovery when the source of ergot is removed.

DRUGS AND CHEMICALS

The poisoning of poultry by drugs and chemicals is most frequently due to accident, carelessness, or the injudicious use of these products as medicinal agents.

Ammonium chloride. This chemical, while not commonly used as a medicinal agent, sometimes has been administered in an effort to prevent ascites or the accumulation of fluids in the abdominal cavity of birds. The nontoxic dose reported by Gallagher (1919) was 15 to 45 grains; the lethal dose was found to be 60 grains.

The clinical symptoms manifested in ammonium chloride poisoning are loss of appetite, depression, progressive weakness, coma, and death. No characteristic lesions can be demonstrated

Arsenic. Arsenical preparations are extensively used in the control and extermination of rodents and insects. When considerable amounts of these arsenical compounds are ingested by birds, toxic reactions may result which frequently terminate fatally. Van Zyl (1929) states that birds are more resistant to arsenic poison-

FIG. 40.1 — Ergot. These black, hard, spindle-shaped masses are the sclerotia of the fungus *Claviceps purpurea* which infects the seed of maturing rye.

FIG. 40.2—Arsenic poisoning in a duck. Hemorrhages in the myocardium and yellow-green necrotic areas in the liver.

velopments are retarded. According to Nunn (1907), when mercury enters the circulation it is not directly eliminated from the body, but it is deposited in most of the tissues, chiefly the liver and kidneys. The lesions found in birds as the result of mercurial poisoning resemble those characteristic of a generalized toxemia. Various degrees of gastroenteritis may be observed throughout the digestive tract with or without distinct hemorrhagic areas. Considerable amounts of greenish gelatinous exudate in the alimentary canal may be sufficient to color the ingested food material. The mucous membranes frequently become necrotic and exfoliate. The kidneys are usually pale in color, showing degenerative changes, and often are studded with minute white foci. The liver shows evidence of fatty degeneration. The abdominal cavity frequently contains a thick viscid fluid greenish in color.

Geese seem to be quite susceptible to poisoning with calomel. A single dose of 2 grains or more may cause death in less than 24 hours. The symptoms and lesions produced in geese are similar to those found in other birds. However, the degenerative changes found in the heart, liver, and kidneys are much more severe

and may account for the rapid and fatal reaction of calomel in geese. Figure 40.3 shows the extensive degenerative changes in the region of the glomeruli of the kidney, and Figure 40.4 shows the formation of crystals in a necrotic focus of the kidney. These changes were observed in the kidneys of experimental geese which were given 2-grain doses of calomel.

Boric acid. Boric acid poisoning is very rare in birds. The practice of using chemicals in the preservation of canned foods in order to inhibit the growth of putrefactive organisms resulted in the addition of boric acid for this purpose. Gallagher (1924) reported that canned string beans to which boric acid was added at the rate of 9 grams per quart were toxic to chickens. The clinical symptoms described were loss of appetite, diarrhea, depression, and progressive weakness, followed by coma and death. The lesions produced were severe gastroenteritis, the mucosa of the crop becoming thickened, necrotic, or gangrenous. Degenerative changes in the kidneys were quite extensive.

Copper poisoning. Copper sulfate and Bordeaux mixture are perhaps the most common copper compounds used in agriculture, the former as a medicinal agent

FIG. 40.3 — Kidney of a goose, showing degenerative changes in region of glomerulus. ×320.

and the latter as an orchard spray. In poultry practice copper sulfate, also known as bluestone or blue vitriol, has occasionally been recommended for the medication of drinking water. If sufficient amounts of this chemical are ingested, fatal intoxication is frequently observed. Gallagher (1919) found that 20 grains of the crystalline salt or 15 grains in solution was the toxic and lethal dose for the fowl.

Pullar (1940a) reported the minimum lethal dose in grams per kilogram of body weight to be 0.9 copper sulfate crystals, 0.3 to 0.5 copper sulfate when mixed with twice its weight in sodium chloride, and copper carbonate 0.9. Both copper sulfate
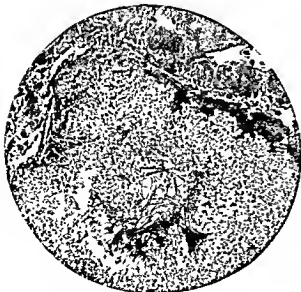
FIG. 40.4 — Calomel poisoning in goose, showing crystals in necrotic foci of kidney. ×320.

and copper carbonate in quantities of 1.0 to 1.5 grams per kilogram of body weight were considered the minimum lethal dose for pigeons, while that for several species of ducks ranged from 0.4 to 0.9 grams. Pullar (1940b) considered the maximum daily intake of copper carbonate tolerated by birds to be 0.06 gm. per kilogram of live weight for fowls and 0.029 for domesticated mallard ducks. No toxic effects were noted from copper sulfate 1 to 4,000 in drinking water for fowls or domesticated mallard ducks.

According to Lander (1926a), the salts of copper in the stomach form albuminates which are quite soluble and rapidly absorbed. They are conveyed to the various tissues by the blood stream and deposited chiefly in the liver, lungs, and kidneys. The elimination of these products in the bile and urine is rather slow. Clinical symptoms of copper poisoning in fowls depend largely on the amount of the toxic agent absorbed. In mild cases a slight depression may be observed, followed by recovery. In fatal cases, a primary stimulation and activity may be noted, followed by severe depression and weakness. Coma, convulsions, and paralysis may occur before death. The lesions consist of catarrhal gastroenteritis accompanied by the secretion of a greenish seromucous exudate. Coagulation necrosis of the mucous membranes of the lower esophagus and crop are often observed. Hemorrhages are frequently found in the mucosa of the intestines. Degenerative changes in the liver and kidneys may be quite severe.

Cresol poisoning. Bullis and Van Roekel (1944) reported on injury and mortality in chicks caused by exposure to fumes from coal-tar creosote oil. Bressler et al. (1951) studied the effect of salt and carbolineum in the experimental production of ascites in turkey poults. Subsequently, West (1957) reported on disinfectant poisoning in chicks. Bierer et al. (1963) studied the effect of feeding 1 per cent coal tar and other disinfectants to chicks for a two-week period.

Under natural conditions, cresol poisoning in chicks occurs most commonly at 3 to 6 weeks of age. The affected chicks are depressed, weak, and have a tendency to huddle. The flock is generally uneven in size and has ragged feathering. Respiratory distress is manifested by rales, gasping, wheezing, and extension of the head and neck. Chicks affected with anasarca may waddle or walk stiff-legged.

The lesions are variable depending upon the severity and duration of the condition. The subcutis on the ventral portion of the body is infiltrated with a yellow transudate. The breast muscles are pale in color. Clear, amber-colored fluid may fill the abdominal cavity. A yellow fibrinous layer may cover the liver. The liver may be enlarged and mottled in early cases or shrunken and cirrhotic in older cases (Fig 40.5). The spleen is pale and small. The kidneys are usually swollen and pale. Hydropericardium is present and the heart may be pale and enlarged. The lungs are often edematous or have areas of hepatization. Fluid and mucus may be present in the trachea. Bullis and Van Roekel (1944) observed blood in the mouth and trachea of some chicks. West (1957) observed that the bone marrow was pale and contained more moisture than normal. He also indicated that the odor of coal tar was detected in the internal tissues of three cases. Bierer et al. (1963) noted a depressed growth in chicks fed a coal-tar disinfectant. He did not observe ascites and edema but but the experiment was terminated in two weeks and sufficient time may not have elapsed for lesions to appear.

West (1957) reported mortality varying from 2 to 56 per cent. He indicated that morbidity and mortality probably depend upon several factors, e.g., the concentration of the toxic agent, overcrowding, type of floor, quantity of litter, prevailing weather conditions, and ventilation.

Further injury may be prevented by moving chicks to new quarters. If this is not possible every effort should be made to improve the ventilation, and the old litter should be removed. Carbolineum

FIG. 40.5 — Cresol poisoning. The liver is gray, shrunken with rounded edges. Ascitic fluid and coagula (arrow) in body cavity.
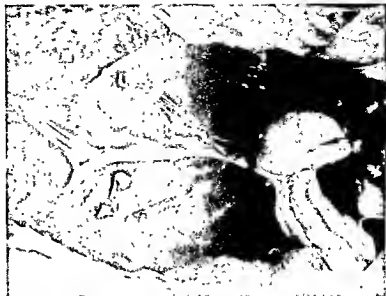
should be used with caution, and the house should be heated and ventilated for several days to dissipate the toxic vapors before the chicks are brooded.

In making a differential diagnosis of cresol poisoning the possibility of salt poisoning, "toxic fat," crotalaria poisoning, and alimentary exudative diathesis must be considered.

These syndromes resemble one another in their clinical manifestations and it is exceedingly difficult for the diagnostician to establish a specific diagnosis. As the etiology of these conditions place responsibility and liability on different parties it becomes mandatory that supportive evidence can be offered in defense of a diagnosis. In those conditions where the responsibility rests with the flock owner, such as cresol poisoning, admission of guilt is more readily elicited by indirection rather than by accusation. The diagnosis of the other syndromes is discussed under their respective headings.

Cyanides. Hydrocyanic acid poisoning may arise not only through the use of the acid and its salts, but through the consumption of certain species of plants which under certain circumstances generate sufficient hydrocyanic acid to cause fatal cases

of toxemia. The highly toxic properties of these compounds make them quite effective in the extermination of rodents, birds, and plant parasites.

Birds are seldom poisoned by cyanides except through accident or carelessness. As a rule, they do not select cyanogenic plants for food except in rare cases when there is no other green feed available. Calcium cyanide is extremely toxic for birds. This compound is used extensively to destroy large numbers of undesirable birds such as sparrows or starlings. This preparation is distributed by dusting machines at night while birds are at roost. The inhalation of the calcium cyanide results in the destruction of the birds in a very short time. Winchell (1925) reported the successful use of calcium cyanide in the destruction of large numbers of domesticated fowls condemned as control measures in an outbreak of European fowl plague. One pound of this compound was sufficient to destroy 2,000 birds within 1 or 2 minutes.

Gallagher (1919) found the toxic dose of potassium cyanide to be 1/10 to 1/2 grain, while the lethal dose was considered to be from 1 to 2 grains. The symptoms develop soon after the consumption of

the toxic substance. The bird usually loses its sense of balance, drops to the floor in a comatose condition, and dies in a very short time. The action of cyanides in large doses is so rapid that characteristic lesions are not well developed. The comb and wattles appear cyanotic; the internal organs become congested; the blood is dark and has an oily appearance; bubbles of gas may be seen in the cavities of the heart. Characteristic odors resembling those of bitter almonds can be detected in the blood and congested organs.

Lead. The commercial preparations of lead which may be responsible for lead poisoning include the oxides and carbonates of lead, lead acetate, lead arsenate, and metallic lead. The oxides and carbonates are used in paint preparations. Lead acetate is used in commercial and medicinal products. Lead arsenate is incorporated in orchard and garden sprays while metallic lead, especially in the form of shot, has been responsible for lead poisoning in fowls and game birds. Large numbers of ducks die each year from ingesting lead shot that contaminate shooting preserves and hunting grounds. In an effort to decrease the number of pellets eaten, attempts have been made to develop shot from an alloy that would gradually disintegrate in the alkali marshes. Most of the lead compounds are comparatively insoluble in water but may become more soluble in acid or alkaline solutions. Lead salts in contact with digestive fluids may form albuminates and other more soluble compounds which are readily absorbed and distributed throughout the tissues by the blood stream. Lead compounds may be deposited in various amounts in the liver, kidneys, bones, and nerve and muscle tissue. Elimination of lead from the tissues is slow and is effected through the bile, urine, salivary, mucous, and cutaneous secretions.

Species. Lead poisoning has been diagnosed in wild mallard ducks, domestic ducklings, Canada geese, guinea fowl, pigeons, and chickens (Wetmore, 1922; Jones, 1939; Wickware, 1940; Stiles, 1940;

Rae and Crisp, 1954; Adler, 1944; Costigan, 1940; Hanzlik and Presho, 1923; Salisbury and Staples, 1958; and Shillinger and Cottam, 1937).

Signs and lesions. Costigan (1940) observed leg weakness and leg paralysis in guinea fowl poisoned by ingesting lead shot. Salisbury and Staples (1958) reported lead poisoning in chicks caused by feeding a grit composed of "frit," an ingredient used in the manufacture of enamelware. Analysis of the "lrit" revealed that it contained 32 per cent lead oxide. Four hundred chicks died out of a flock of 600 during the first two weeks of age. They reported that the most consistent lesions of lead poisoning in the chicks were submucosal hemorrhage and necrosis of the gizzard lining. Experimental poisoning of adult White Leghorns was followed by loss of weight, cessation of egg production, and green diarrhea in later stages. The birds had severe anemia but no evidence of stippling was found on blood smears. All birds had necrosis of the gizzard lining. Johns (1934), in a study of lead poisoning in ducks, considered the stippling of red blood cells as characteristic of a dying cell, and that the selective affinity of lead salts for immature red blood cells caused their early destruction.

Coburn et al. (1951) reported that they were unable to find evidence of stippling in blood cells due to lead poisoning in any avian species. These workers did observe poikilocytosis and anisocytosis in ducks experimentally poisoned with lead nitrate. Abnormal red cell shapes included dumbbell, bottle, oat, sickle, and teardrop forms. In chickens that are anemic from lead poisoning, enucleated erythrocytes may be found in blood smears (Fig. 40.6).

Toxic levels. Wetmore (1922) reported that the usual number of shot found in dead waterfowl was 15 to 40. The maximum number of shot recorded was 150, and the average was 25. Hanzlik and Presho (1923) observed clinical symptoms in pigeons 8 to 10 days after the introduction of lead shot directly into the crop.

FIG. 40.6 — Lead poison-
ing. Blood smear from a
chicken. Note the enu-
cleated, spindle-shaped
erythrocyte and the tear-
drop-shaped erythrocyte.
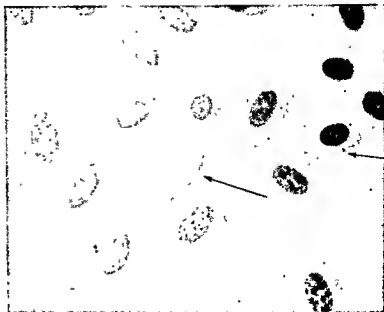
They considered the minimum lethal dose
to be 0.16 gram of metallic lead per kilo-
gram of body weight. Coburn et al. (1951)
determined the critical daily dosage of
lead to be between 6 and 8 mg/kg of body
weight when lead was given as an aqueous
solution of lead nitrate. At the higher
level the survival period was about 4 weeks.
McIntosh and Staples, quoted by Salisbury
and Staples (1958), failed to produce any
gross evidence of lead poisoning in fowl
by feeding single massive doses of red
lead and white lead in amounts up to 1,000
mg/kg of body weight.

Diagnosis. The signs and lesions of lead
poisoning are not pathognomonic and sup-
portive evidence is needed before a posi-
tive diagnosis can be made. Finding metal-
lic lead in the digestive tract in conjunc-
tion with a high lead content in the liver
or bones would establish a positive diagno-
sis. Adler (1944) investigated lead poison-
ing in Canada geese and found there was
no correlation between the amount of lead
in the leg bones and the number of shot
found in the gizzard. He stated that the
amount of lead found in the leg bones is
a measure of the duration of exposure to
the lead, as only a small portion of the

daily intake of lead is stored, the rest
being excreted. He did find, however,
that there was a correlation between the
lead content of the liver and the number
of shot present in the gizzard. From these
findings he concluded that the best organ
for chemical analysis to confirm a diagno-
sis of lead poisoning was the liver. A high
lead content in the liver would indicate
that the lead was recently ingested whereas
a high lead content in the bones would in-
dicate chronic poisoning.

Naphthalene. Naphthalene formerly was
frequently used in the form of moth balls
as a protection against lice and mites in
nests. This preparation is fairly volatile
and gradually decreases in volume on ex-
posure to air. When the moth balls be-
come small they may be readily ingested
by fowls. The loss of 40 fowls in a flock
of 400, caused by naphthalene poisoning,
was reported by Hudson (1936). The
clinical symptoms associated with this
form of poisoning include congestion of
the comb and wattles, abnormally bright
eyes, greenish-black diarrheal excrement,
progressive paralysis, and death. Death
usually occurs within 3 days following the
first appearance of diarrhea. The lesions

consist of severe catarrhal gastroenteritis with necrotic areas in the mucous membrane of the crop. The liver is congested and greatly enlarged, with numerous small necrotic foci. The strong characteristic odor of naphthalene can be detected in the contents of the crop and gizzard.

Nicotine sulfate. Nicotine, the highly toxic alkaloid of tobacco, has been used for many years by nurserymen and gardeners for the control of insects. According to Carpenter (1931), the development of the commercial "Black Leaf 40," a standardized 40 per cent solution of nicotine sulfate, made it possible to standardize the dosage and obtain more efficient results in the treatment of internal parasites of poultry. Apparently mature fowls tolerate greater doses of nicotine sulfate than do mammals or other animals. Various commercial preparations containing nicotine sulfate and other constituents, including kamala, have been used with varying degrees of success in controlling intestinal parasites. Bleecker and Smith (1933b) reported toxic reactions in birds treated internally with "Black Leaf 40." They (Bleecker and Smith, 1933a) also reported the toxic dose of this preparation to be from 0.5 to 1.0 cc. In some cases birds receiving a toxic dose became depressed and prostrated and died in a short time. Parker (1929) found that baby

chicks were quite susceptible to poisoning with nicotine sulfate. Doses of 0.2 cc. in various concentrations were given with the following results: an 8 per cent solution killed all treated chicks; 6 per cent solution was fatal to 70 per cent; 4 per cent solution resulted in the loss of 50 per cent; and 3 per cent solution, though producing a toxic reaction and coma for about 15 minutes, resulted in very few deaths.

The application of nicotine sulfate in the form of "Black Leaf 40" on the roosts of the hen house when fowls go to roost has been quite effective in controlling external parasites. According to Carpenter (1931), nicotine is highly volatile at 100° to 105° F. and is volatilized by the body temperature of the fowl. Cases of severe intoxication have been reported from the improper use of this product. Proper ventilation of the poultry house prevents the possible accumulation of vapors sufficient to produce toxic reactions.

The symptoms observed in nicotine poisoning include severe depression, retarded respiration, cyanosis, and coma followed by death. The lesions usually observed are as follows: congestion of the lungs and liver, ecchymoses of the lungs and heart, congestion of the nictitating membranes, dilatation of the pupil (Fig. 40.7), and a dark cyanotic condition of the blood.

FIG. 40.7 — Nicotine sulfate poisoning in chickens, showing dilatation of the pupil.

15 mg. per kilogram of live weight. The repeated ingestion of sublethal doses may result in chronic toxemia.

Postmortem examination reveals various degrees of congestion with the accumulation of some serous fluid in the pericardial sac as well as in the abdominal cavity in some cases. Enteritis is usually limited to the upper part of the small intestine. A characteristic pungent odor of phosphorus can be detected in the contents of the crop and gizzard especially in birds which have ingested large doses.

Nitrates. The nitrates of potassium and sodium have been known to produce poisoning in poultry, the general character of which is similar to that of sodium chloride poisoning. Sodium nitrate is commonly used in the form of Chile saltpeter as a fertilizer and may be mistaken for sodium or magnesium sulfate. In attempted medication of fowls, such errors have occurred with fatal results. Guberlet (1922) reported the lethal dose to be from 60 to 70 grains for the average fowl, smaller doses causing digestive disturbances accompanied by diarrhea.

The clinical symptoms most frequently observed are excessive thirst, anorexia, vomition, diarrhea, retarded heart action, subnormal temperature, and cyanotic appearance of comb, wattles, and skin. Muscular weakness develops into progressive paralysis followed by coma and death. In some cases convulsions appear shortly before death. The lesions include varying degrees of gastroenteritis, frequently of a hemorrhagic nature. Degenerative changes may be observed in the heart, liver, and kidneys. In peracute cases the lesions are less distinct.

Potassium permanganate. This chemical compound is frequently used as an antiseptic in drinking water for poultry but is decidedly toxic if administered in greater amounts than recommended for therapeutic use. Mature fowls are apparently not injured by consuming a 1:500 solution of potassium permanganate as drinking water for several weeks. Gallagher (1919) reported the toxic dose to

be 30 grains. He also reported that an experimental fowl died in less than 24 hours following the administration of the toxic dose. No clinical symptoms were observed prior to death. The lesions consisted of a severe cauterization of the crop wall. The submucosa and skin on the lower surface of the crop were blackened. Extensive blood clots were found in the crop where the tissue came in contact with the chemical crystals. All the other organs were normal. The potassium permanganate apparently did not leave the crop, the caustic action of the chemical compound being localized in the tissues with which it came in contact.

Sodium bicarbonate. Years ago it was a common practice for poultrymen to "flush" their flocks with various chemicals in the drinking water, one of which was sodium bicarbonate. Pathologists in diagnostic laboratories observed that chicks with lesions of visceral gout often had a history of receiving sodium bicarbonate as a flush. Subsequent investigation revealed that overdosing with sodium bicarbonate caused nephritis and visceral gout (Delaplane, 1934).

Witter (1936) reported toxic reactions but no mortality in 2-week-old chicks which were given an 0.6 per cent solution of sodium bicarbonate. However, a level of 1.2 per cent sodium bicarbonate caused toxic reactions in 2 days and mortality by the fourth day. Chicks 6 to 8 weeks of age which were given a 2.0 per cent sodium bicarbonate solution became sick in 2 days and began dying by the third and fourth days. Administration of a 2.4 per cent solution to yearlings produced toxic symptoms and death in five days.

Clinical signs included depression, weakness, increased water consumption, and watery droppings. The primary lesion occurred in the kidneys which became pale and swollen initially, followed by marked distension of the tubules and ureters with urates. Urate deposits were found on the epicardium and surfaces of liver and lungs.

Jungherr (1935) reported that single doses or repeated small doses of sodium bi-

carbonate may cause visceral gout. He also stated that visceral gout may occur in 2-day-old chicks that had not received feed or medication of any kind. Bullis and Van Roekel (1944) observed visceral gout in chicks 3 to 4 days of age that had not received sodium bicarbonate. Losses in these cases were usually less than 5 per cent. They also stated that excess sodium bicarbonate fed to chicks would produce visceral gout (Fig. 40.8).

Scrivner (1946) reported upon the effect of various concentrations of sodium bicarbonate in the drinking water of day-old poults. Levels of 0.1 per cent sodium bicarbonate and below were nontoxic when given for 18 days. Sodium bicarbonate at concentrations of 0.3, 0.5, and 0.6 per cent produced 20, 60, and 70 per cent mortality, respectively, in 2 to 3 weeks. It should be noted that the lesions in the dead poults were subcutaneous edema and ascites. None of the dead poults had visceral gout whereas the reports of sodium bicarbonate

poisoning in chicks describe gout as one of the principal lesions.

Other sodium compounds. Scrivner (1916) studied the effect of various sodium compounds on day-old turkey poults when administered in the drinking water at a concentration of 0.75 per cent. Administration of sodium citrate killed 19 out of 30 poults in 22 days. Lesions produced were subcutaneous edema and ascites. Sodium iodide administered for 5 days killed 100 per cent of the poults, 2 of which had lesions of ascites and subcutaneous edema. Sodium sulfate administered for 15 days killed 11 out of 31 poults with lesions of ascites and subcutaneous edema. Sodium hydroxide, administered at a concentration of 0.1 per cent in the drinking water for 21 days killed 2 poults which had ascites and subcutaneous edema. From the foregoing it is apparent that sodium compounds other than NaCl and sodium bicarbonate may be toxic for birds and produce lesions of ascites and edema.

FIG. 40.8 — Sodium bicarbonate poisoning. Five-day-old chick with urate deposits over the viscera and muscles.

Sodium chloride. It has been shown that most species of domestic fowl such as the turkey, chicken, duck, and pigeon are susceptible to salt poisoning. Young birds are more susceptible than mature birds which is probably the result of young birds consuming more feed in relation to body weight. Salt poisoning could arbitrarily be classed as acute or chronic. The acute cases would be those in which the birds consumed large amounts of salt in a short period of time. This could happen when birds were accidentally exposed to large amounts of salt, such as rock salt used in chilling brine, fish brine, or mixed with sand for highways. Gallagher (1919) reported 2.5 drams of this salt to be the toxic dose for chickens. The chronic cases of salt poisoning are more common than the acute and occur where there is salt in the drinking water or excess salt in the feed. Bigland (1950) reported on field cases of salt poisoning in turkey poults and found that in some instances the feed contained an amount in excess of 1 per cent salt over what the manufacturer intended. In addition, some of the water samples contained salt. He also found that the salt content in the feed hoppers was higher than in feed taken from the bag. Apparently, the salt by virtue of its specific gravity and physical characteristics gravitates to the bottom of the feed hopper. Losses started at 8 to 9 days of age with a daily mortality of 1 to 3 per cent continuing to 14 to 18 days of age. Total losses amount to 10 to 20 per cent.

The clinical symptoms include loss of appetite, severe depression, and progressive paralysis, followed by respiratory failure and death. In poults at necropsy, Bigland (1950) found anasarca, ascites, hydropericardium, cardiac hypertrophy, edema of the lungs and enteritis in the duodenum with edema of the intestinal wall. Increasing the salt in the diet causes a marked increase in water consumption and this was noted in experiments by Krakower and Goettsch (1945) and Paver et al. (1953). The additional stress placed upon the kidneys by increased salt and water intake

is reflected in enlargement of the kidneys with glomerular hypertrophy.

Young poults are much more susceptible than older turkeys. Bressler et al. (1951) noted that considerable mortality in young poults was produced when 0.9 per cent or higher levels of salt were added to the ration. Ewing (1947) observed that a 2 per cent level of salt produced 38 per cent mortality in turkey poults. In one trial, Scrivner (1946) fed 2.5 per cent salt in the mash and produced 40 per cent mortality in poults at 7 days of age accompanied by lesions of edema and ascites. However, Roberts (1957) using turkeys of 8 to 31 weeks of age found that salt levels up to 6.0 per cent in the ration increased the water intake but did not affect the growth and development. A reduction in weight gain was noted where 6.0 and 8.0 per cent salt was fed but no mortality occurred. Scrivner (1946) reported that feeding 2.0 per cent sodium chloride in the water and 0.5 per cent salt in the feed to day-old poults produced depression in 48 hours and 100 per cent mortality in four days. There was no edema or ascites. When the concentration of salt in the water was reduced to 1 per cent with 0.5 per cent in the feed all poults died in five days with lesions of ascites and edema. Lowering the salt concentration in the water to 0.5 per cent in addition to feeding 0.5 per cent salt in the mash caused 80 per cent mortality in 10 days with lesions of subcutaneous edema and ascites.

Paver et al. (1953) found no harmful effect in feeding day-old chicks salt levels ranging from 0.98 to 3.25 per cent in the mash. Levels of salt above 3.5 per cent did cause mortality in chicks. They found that a gradual increase in the salt concentration of the diet fed to chickens over one month of age from 2 per cent to 50 per cent interfered greatly with growth but was not necessarily fatal. It was postulated that this was due to the lower food requirements of adult birds in relation to live weight and possibly increased renal efficiency in older birds.

Gordon et al. (1959) reported the pro-

duction of a condition resembling "toxic fat" disease in 2 to 6 per cent of 4-week-old chickens fed purified diets containing 20 per cent blood meal, 2 per cent corn oil, and 0.88 per cent sodium chloride. Symptoms and lesions were not produced in chickens fed similar diets containing 30 per cent blood meal and 22 per cent corn oil. When ether-extracted blood meal was fed with the high level of NaCl no edema resulted. Supplementation of isolated soybean protein diets with high levels of NaCl resulted in edema only in chickens fed diets low in fat. It was concluded that there is an ether-soluble factor present in blood meal which disturbs salt regulation of the young chicken. In addition, high levels of fat can counteract the edematous effects of a high dietary NaCl level

Shaw (1929) reported that less than 5 grams were nontoxic for ducks weighing 600–800 grams, while larger doses were lethal. Torrey and Graham (1935) reported four consecutive doses of 5 to 6 grams of salt were fatal to half-grown Pekin ducks. However, the ducks tolerated doses of 1 to 2 grams of sodium chloride daily for 29 days. The clinical symptoms of toxicity were depression, loss of appetite, incoordination, progressive weakness, prostration, and death. Lesions varied from a mild congestion of the duodenum to severe enteritis accompanied by nephritis.

Edwards (1918) investigated the toxicity of sodium chloride for pigeons and reported that feeding more than 3 grams per kilogram of body weight produced toxic reactions and above 3.3 grams was fatal. The symptoms and lesions were similar to those in other birds. According to Buckley et al. (1939) there is no antidote for sodium chloride poisoning. However, every effort should be made to provide the birds with readily accessible clean fresh water and a salt-free diet.

Kamala. Kamala must be regarded as a poison even though it is used as an anthelmintic for the removal of tapeworms in poultry. It is a powerful irritant in the gastrointestinal tract. Care must be used in the administration of kamala in order to avoid toxic reactions. Following flock treatment with kamala, the egg production invariably is reduced for some time. Hall and Shillinger (1926) considered a 15 grain dose as an effective anthelmintic for mature fowls. Turkeys are less tolerant to kamala than chickens according to the report of Beach (1930). Hawn (1933) found kamala was neither a safe nor an efficient anthelmintic for turkeys. Cram (1928) warns against the use of kamala in birds affected with complicating diseases because of resulting high mortality.

Strychnine. Strychnine is a powerful toxic alkaloid occurring in the seeds of certain species of the Loganiaceae. It is readily absorbed from the digestive tract into the blood stream and is a powerful stimulant to the central nervous system. Its elimination from the tissues is slow, thus intensifying the cumulative action. Toxic doses produce tetanic spasms, paralysis, respiratory failure, and death. Strychnine is used extensively in the control of rodents. Accidental strychnine poisoning occasionally occurs in animals and birds consuming poisoned baits. Fowls apparently are more resistant to strychnine than mammals. According to Heinekamp (1925), the toxic dose in fowls depends largely on the quantity and nature of the crop contents, the absorption of the toxic agent being inversely proportional to the amount of food in the crop and directly proportional to its fluidity. Gallagher (1919) regarded 0.08 gram per kilogram of body weight as the lethal dose for chickens.

Sulfonamides. Sulfonamides were among the first of the so-called "wonder drugs" to emerge following World War II. Their success in the treatment of human diseases led to experimentation in the field of veterinary medicine. The therapeutic level and toxic level of some sulfonamides may impinge on one another and caution is needed in regard to dosage and duration of treatment. The small amount of the drug used in proportion to the bulk of the

the feed makes thorough mixing and distribution throughout the feed difficult. The ready availability and indiscriminate use of medications by poultrymen may lead to cases of intoxication. Levine (1939) in studying the effect of sulfanilamide against coccidiosis found that concentrations of 0.2, 0.3, and 0.4 per cent by weight of mash fed for a period of two weeks was definitely toxic for chickens. Farr and Wehr (1945) fed sulfamerazine to chickens and produced necrosis of the liver and spleen as well as retarded weight gains. Levine and Barber (1947) found hemorrhagic infarcts, necrosis and swelling of the spleens in chickens following the feeding of three sulfa drugs. Delaplane and Milliff (1948) reported that laying pullets which were fed sulfaquinoxaline in the mash at a level of 0.25 per cent for a period of eight to ten days rapidly declined in egg production, became droopy, weak, anemic, and eventually died. Davies and Kendall (1953) and Davies (1954) found that sulfaquinoxaline added to the drinking water in a concentration of 0.0645 per cent produced a toxic effect after treatment for only 5 days.

Yacowitz, et al. (1955) reported that the feeding of 0.1 per cent sulfaquinoxaline in the presence of 3–5 per cent alfalfa and 5 mg. of menadione per pound of feed resulted in the occurrence of a hemorrhagic syndrome in chicks. Hemorrhages closely resembled those encountered in field cases of hemorrhagic disease. The feeding of iodinated casein and penicillin appeared to increase the toxicity of sulfaquinoxaline. The whole blood clotting time was increased but the prothrombin time was normal.

Marthedal and Velling (1961) indicated that macroscopic changes were produced in five to eight-week-old chickens in four days following the administration of 0.05 per cent sulfaquinoxaline in the drinking water. From these reports it is apparent that the various sulfonamides may be toxic under certain conditions.

Signs. Toxicity is manifested in growing birds by ruffled feathers, depression,

and a pale or icteric color of the tissues about the head. Farr and Jaquette (1947) noted that birds treated with sulfamerazine in the mash at levels higher than 0.25 per cent made poorer weight gains due to the unpalatability and toxic effect of the drug. In laying flocks, toxicity is manifested by a marked drop in egg production. Edema and hemorrhage of the wattles may occur (Fig. 40.9). Scott et al. (1944) studied the effect of feeding sulfanilamide to birds in production. They indicated that sulfanilamide in the diet would cause decreased production, thin shells, rough shells, and loss of pigment in the shell. In controlled studies they found that the addition of 0.008 to 0.5 per cent sulfanilamide to the diet caused an immediate effect on shell quality. Egg production practically ceased at levels above 0.25 per cent. It was also determined that blood calcium levels were not depressed by sulfanilamide feeding. The thinness of the shell could not be attributed to premature expulsion of the egg as it remained in the shell gland for the normal length of time.

Gross lesions. Levine and Barber (1947) and Delaplane and Milliff (1948) reported that spleen lesions varied from hemorrhagic infarcts to small whitish foci (Fig. 40.10). In a few instances more than half the spleen was a white necrotic mass. The liver was frequently involved with greyish-white nodules about 1 mm. in diameter

FIG. 40.9 – Sulfonamide poisoning in an adult chicken. Note depressed attitude and swollen hemorrhagic wattles.

FIG. 40.10 — Hemorrhages in myocardium and small white granulomas in the spleen due to sulfonamide intoxication.

scattered throughout the tissue (Fig. 40.11). Similar greyish-white nodules and petechiae occurred in the kidney. Nodular lesions were also present in the myocardium and lungs. Farr and Jaquette (1947) noted that the spleen was the organ most frequently affected in sulfonamide intoxication. Delaplane and Milliff (1948) also found subcutaneous hemorrhages. Yacowitz et al. (1955) reported that lesions following the feeding of sulfaquinoxaline were watery blood, yellow fatty bone marrow, and hemorrhages in the thigh and breast muscles (Fig. 40.12). Hemorrhages were present in the heart, and the liver and intestine contained numerous petechial and ecchymotic hemorrhages (Fig. 40.13).

Histopathology. Caseation necrosis, giant cells, lymphocytic and eosinophilic infiltration were observed in the liver by Dela-
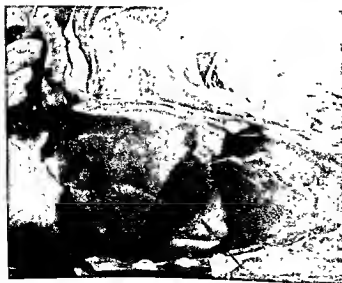
FIG. 40.11 — Sulfonamide intoxication in a chicken. Small granulomas in the liver (arrow), and a pale reticulated network along the edges of the liver.
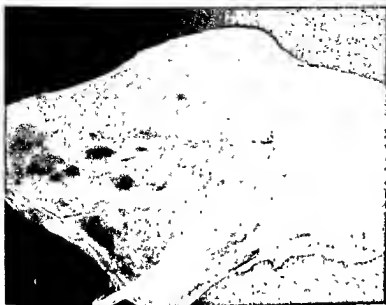
FIG. 40.12 — Sulfonamide poisoning. Hemorrhages in the breast muscle of a chicken.

plane and Milliff (1948). In the absence of caseation there was hyperplasia of the connective tissue stroma. Nearly every follicle of the spleen contained multinucleated or giant cells. Some follicles had caseation necrosis and infiltration of eosinophils. The lungs had small circular tuberclelike areas composed of a caseous center surrounded by giant cells. Eosinophils were numerous in the center of

nodules that were not caseated. Lymphocytes and neutrophiles infiltrated the periphery of the nodular areas. The kidneys had hemorrhages and nodular areas composed of lymphocytes, granulocytes, and giant cells.

Alpha naphthyl thiourea. This preparation, commercially known as ANTU, which was recently developed as a rodenticide and is one of the most effective agents
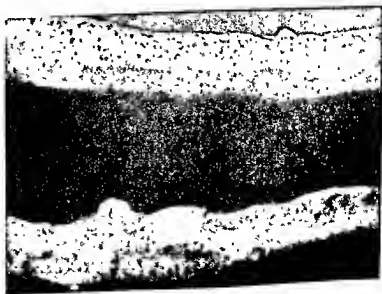
FIG. 40.13 — Sulfonamide poisoning. Punctate hemorrhages in the mucosal surface of the intestine of a chicken.

for this purpose, is also highly toxic for domesticated animals and poultry. While there is much critical work to be done in determining the toxicity and lethal dosages of this product, Anderson and Richter (1946), through their experimental work with chickens, found that young chicks are quite susceptible to poisoning by this preparation. It is apparently less toxic for older birds. Chicks ranging in age from 3 to 5 weeks and fed 2 per cent to 3 per cent ANTU in mash showed toxic reactions in a short time. Half of these birds were dead in 18 hours. Most of the survivors which were fed plain mash recovered and those which continued to be fed on the toxic ration ate very little and died within 4 days. The clinical symptoms include depression, loss of appetite, listlessness, incoordination, weakness, prostration, and death. Well-defined pathologic lesions were not developed in all the poisoned birds. There was evidence of edema in the lungs and excessive quantity of fluid in the pericardial sac. Some cases showed evidence of fatty degeneration of the liver and kidneys.

Pullets averaging 1½ pounds were given doses up to the quantity to be found in 6 ounces of 2 per cent poisoned mash. Some died, and the survivors lost weight and would have been unprofitable birds if raised to maturity. Pulmonary edema, fatty degeneration of the liver and kidneys, and in some cases degeneration of the heart constituted the principal pathologic changes.

Sodium monofluoroacetate. This chemical compound, commonly designated as "Compound 1080," is one of the most effective rodenticides recently developed. It is effective in the extermination of rodents but is also toxic for domesticated animals and birds. Cottral et al. (1947) determined that the minimum lethal dose for chickens is about 14 mg. per kilogram of body weight and that repeated sublethal dosages will produce death in a short time. The clinical symptoms include restlessness, followed by a definite heart acceleration and increased respiration. Sublethal dosages

frequently produce a definite blanching of the comb and wattles, while heavier dosages will induce congestion and cyanosis of those appendages. As the toxemia progresses, the birds become weak and comatose. Some cases develop nervous symptoms and convulsions prior to death. The pathologic changes observed in this type of poisoning include distention of the pericardial sac with clear straw-colored fluid, hemorrhages in the cardiac tissue as well as on the endocardium, severe degeneration of the heart tissue, dark tarlike color of the blood, and large quantities of serous straw-colored fluid in the lungs and thoracic cavity. The liver appears dark in color, and the gallbladder is distended with bright green, watery bile. Degenerative changes in the kidneys may occur in some cases as well as a mild enteritis. The toxic action is primarily on the heart, and no effective treatment for this condition is known at the present time.

DDT (dichloro-diphenyl-trichloroethane). Dry DDT crystals and the water-dispersible preparations used in reasonable quantities as indicated for their use in insect control are incapable of causing toxic reactions in domesticated poultry. From the results of experimental studies, it would appear that great quantities of 5 per cent to 10 per cent DDT preparations would have to be ingested, inhaled, or dusted upon the bird to cause an unfavorable reaction. This would be a far greater quantity than that to which poultry is likely to be exposed under normal conditions. According to Kingscote and Jarvis (1946), the oil preparations are regarded as more dangerous, but not sufficiently so as to exclude their use if reasonable care is exercised. Their primary use is for controlling gnats, flies, and some intermediate hosts of poultry parasites. McNeil and Hinshaw (1947) reported that water emulsions were more effective than kerosene emulsions and safer to use. There apparently was no advantage in using concentrations greater than 2 lb. per 100 gal. of water. The amount recommended is 5 gal. per 500 sq. ft., resulting in the dis-

persion of about 100 mg. of powder per square foot.

The clinical symptoms observed in DDT poisoning include dyspnea, rapid breathing through the mouth, rapid blinking of the eyelids, muscular spasms, progressive incoordination, prostration, and death. Young birds may die in a short time without showing any well-defined clinical symptoms. There are no characteristic gross pathologic changes in the internal organs except the heart, which frequently shows petechial hemorrhages on the base. The majority of the birds show no specific postmortem lesions which can be of diagnostic value.

Arasan poisoning. Arasan [tetramethylthiuram disulfide (TMTD)], a fungicide used in the treatment of seed corn, was reported toxic to hens and chickens by Johnson et al. (1955), Waible et al. (1955) and Schumacher and Heuser (1956). The major symptoms observed in laying hens include misshapen and soft-shelled eggs, retarded production, and finally completely arrested production. Heuser and Schumacher (1956) reported a drop in production from 70 per cent to 10 per cent in 3 days after feeding a ration containing 33 per cent of Arasan-treated corn. In chicks, reduced growth and development as well as feed conversion efficiency are first observed, followed by hock disorders, inability to stand, and greatly increased mortality.

This product is extremely toxic, and such low levels as 7.5 parts per million of the compound in poultry rations will produce toxic reactions. The similarity of Arasan toxicity symptoms in hens with those produced by mild outbreaks of infectious bronchitis or Newcastle disease makes field diagnosis difficult. Seed corn may be treated with TMTD levels as high as 750 to 1,000 p.p.m., and such levels would make it practically impossible to dilute corn sufficiently to prevent toxic effects in poultry rations. According to Swanson et al. (1956), low levels of Arasan-SFX (75 per cent TMTD) resulted in a reduction in eggshell thickness and

firmness in albumen. At levels above 100 p.p.m., practically no hard-shelled eggs were produced.

Dieldrin. Carnaghan and Blaxland (1957) reported mortality in wild pigeons and pheasants presumably caused by the ingestion of seeds treated with dieldrin. As circumstantial evidence strongly suggested that the birds were eating treated seed, controlled experiments were carried out to determine the toxicity of various chemicals used in seed treatment.

Grain dressings containing organo-mercurials and gamma benzene hexachloride were found to be nontoxic to pigeons and pheasants. The feeding of wheat treated with dieldrin caused mortality in pigeons and pheasants. The administration of washings from 1½ oz. of dieldrin-treated grain proved fatal to adult pigeons. Deaths occurred between 4 and 10 days after administration of the toxic product.

The first symptoms manifested were a listlessness and a "hunched up" attitude. The birds did not maintain an even flight and lost their balance when lighting. Before death, nervous symptoms became pronounced and were characterized by rapid lateral movements of the head with slight tremor of the head and neck. There was constant blinking of the eyelids. Death occurred during a violent convulsion. At necropsy the liver and kidneys were congested. The gizzard lining was degenerated and hemorrhages were present on the surface of the underlying muscle.

Chlordane. Rosenberg and Tanaka (1950) showed chlordane to be definitely toxic to growing chickens. The tolerance of chicks to chlordane poisoning apparently increases progressively in different age groups. The addition of 0.25 per cent chlordane to the ration caused deaths as early as the first and second days in the 7-day-old group. Deaths were recorded after 2 days in the 21-day-old chicks; in 4 days in the 63-day-old birds and in 8 days in the 112-day-old group. All experimental chicks died within 17 days on the ration. Chlordane killed all 7-day-old chicks when fed at levels ranging from

0.10 to 0.25 per cent. The ration containing 0.05 per cent chlordane killed only 66.6 per cent of the chicks. The primary lesions found in all of the fatal cases were in the heart. Excessive quantities of fluid were found in the pericardial sacs; there were enlargement and distortion of the heart as well as engorgement of the coronary vessels.

The toxicity of chlordane to laying pullets was reported by Rosenberg *et al.* (1950). The experimental birds were highly resistant to chlordane poisoning. None of the birds receiving 0.05 or 0.15 per cent chlordane died during a 28-day test, and those receiving 0.25 and 0.5 per cent survived without mortality for 27 and 21 days, respectively. The birds fed 0.5 per cent chlordane completely ceased egg production and went into a molt; feed consumption decreased as did the body weight. Shriveling and cyanotic combs were observed. The group receiving 0.05 per cent of the drug was only slightly affected, continuing to lay nearly as well as the controls, and showed no clinical symptoms. Gross lesions observed in dead birds receiving the lethal doses consisted of heart lesions similar to those described in chicks.

Post (1951) compared the toxic effect of chlordane and toxaphene in pheasants and Chukar partridges. He determined that the toxic level of chlordane was 200 mg. per kilogram of body weight for pheasants and Chukar partridges. The maximum survival time for the pheasants was 56 days at the lower level of drug decreasing to 10 days when a level of 5,000 mg. per kilogram of body weight was given. Partridges died much more acutely than the pheasants with the shortest survival time being 2 hours and the maximum survival time 72 hours.

The minimum lethal dose of toxaphene was 200 mg. per kilogram of body weight for pheasants. Death occurred within 20 days when the minimum toxic level was fed and within 4 hours when levels of 5,000 mg. per kilogram were fed. The minimum lethal dose for partridges was 50 mg. per

kilogram of body weight. Some birds died within 2½ hours after administration of the drug and maximum survival time was 5 days. Poisoning by toxaphene and chlordane produced similar signs and lesions.

Lindauc. Bootes (1962) reported poisoning in a group of 200 turkey poults following the use of lindane in the litter. Approximately 300 grams of an insecticide powder containing 10 per cent lindane was sprinkled over the litter in a 9' × 9' brooder house. About 30 minutes after the application of the insecticide the poults stopped eating and began to squeak noticeably. Shortly thereafter, many of the birds showed signs of nervous derangement, manifesting opisthotonus, flapping of the wings, tetanic spasms of the muscles, and finally lapsing into a coma before death. At necropsy the only lesion was a slight edema in some poults. Within twenty-four hours 75 poults had died. Although the poults were moved to clean litter at the end of twenty-four hours, losses continued for a week and a total of 170 in the original group of 200 eventually died.

Malathion. Gaafar and Turk (1957) studied the toxicity of Malathion for chickens. They determined the LD_{50} of Malathion for 3-week-old chickens to be between 200 and 400 mg. per kilogram of body weight and for yearlings between 150 and 200 mg. per kilogram of body weight. Almost all of the poisoned birds recovered if they survived the first 16 hours. The signs of Malathion toxicity in the poisoned groups were drowsiness, incoordination, reluctance to move, resting on their hocks, excessive salivation with mucus hanging from the beaks, cyanosis of the skin, diarrhea, blood-tinged brownish droppings, coma, and death. At necropsy the principal change was congestion and discoloration of the heart muscle. Many birds had a marked dilatation of the subcutaneous blood vessels. These workers also reported that no signs of toxicity resulted when chickens were dusted at weekly intervals for four weeks with a 4 per cent Malathion powder.

Golz and Shaffer (1955) reported that there was no evidence of toxicity in chickens after they were fed mash containing 100 and 1,000 p.p.m. of Malathion for ten weeks. At a level of 5,000 p.p.m. of Malathion in the mash or an average consumption of 450 mg. per kilogram of body weight per day these investigators found that chickens showed definite signs of toxicity such as retarded growth, poor feathering, soft droppings, weakness of the legs, and paralysis.

Chlorinated hydrocarbon. McCune et al. (1962) reported hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Investigation of this compound was prompted by the observation that chicks reared in a battery freshly painted with an epoxy-resin paint developed ascites and hydropericardium similar to the lesions found in the "toxic fat" syndrome. Various fractions of the paint were mixed with the mash and it was found that the major toxic product was chlorinated biphenyl which was added to the paint as a plasticizer.

Feeding 0.04 per cent biphenyl in the feed produced symptoms and mortality starting at 3 weeks of age and reaching major proportions during the fourth week. Affected chicks had labored respiration and rales. At necropsy the abdominal cavity was distended with fluid and hydropericardium was present. The crops of some birds contained bloody fluid. The kidneys were swollen and pale in most chicks but in the advanced stages many were hemorrhagic. Some livers were enlarged and mottled in appearance. The lungs were commonly hydropic and hemorrhagic. A yellow gelatinous transudate was frequently found under the skin and within the body cavity.

Thiophosphate poisoning. A commercial thiophosphate spray (Diazinon) used for insect control proved to be highly toxic for White Pekin ducks. The youngest birds, 8 and 15 days old, suffered 100 per cent mortality 1 hour after the spraying operations were completed. The mortality of the older birds, 22 to 36 days of age,

was 50 per cent in 5 hours and an additional loss of 25 per cent within the next 24 hours.

The only clinical manifestations observed were inability to stand and tremors of the head and neck. Pathologic tissue changes were confined to acute congestion of the lungs. According to Dougherty (1957), diagnosis by lesions or clinical laboratory tests is difficult if not impossible.

The lethal dose per os for Malathion, a less toxic thiophosphate preparation, is 1,100 mg. per kilogram and for Diazinon is 14 mg. per kilogram in White Pekin ducklings under experimental conditions. The intratracheal lethal dose is 600 mg. per kilogram for Malathion and 6 mg. per kilogram for Diazinon. Insecticides considered safe for chickens or other gallinaceous birds may be highly toxic for other species.

Carbon monoxide. Severe losses in chicks and turkey poults may be caused by carbon monoxide poisoning, primarily due to poorly ventilated brooders or the result of defective coal or oil heating units. Adequate ventilation of brooders and housing facilities is an important factor in poultry management. Accumulation of carbon monoxide may prove fatal to entire units of young birds before the condition is discovered. The symptoms of acute carbon monoxide poisoning include restlessness, drowsiness, stupor, labored breathing, and incoordination. As the toxemia progresses, the birds gasp, fall, and lie on their sides with heads thrown back. They commonly develop spasms or convulsions prior to death. Many acutely affected birds recover when removed to fresh air. In subacute cases, the feathers may appear rough, the appetite is diminished, and evidence of nutritional disturbances is manifested by retarded development and growth. Stiles (1910b) found that 0.04 to 0.05 per cent carbon monoxide is sufficient to produce definite toxic reactions. The principal lesion observed in acute carbon monoxide poisoning is the bright cherry-red color of the lungs and blood. The

only from about July first to the middle of August. He reported losses in mature fowls following the ingestion of black locust leaves. The toxic material produces a severe hemorrhagic enteritis, followed by depression, paralysis, and death in 12 to 24 hours.

Corn cockle. The corn cockle (*Argostemma githago*) is a weed which grows in wheat fields throughout the world. The seeds of this plant are highly toxic. The whole cockle seed is very unpalatable and is usually avoided by birds, but in ground grain mixtures it may be consumed in sufficient quantities to produce a fatal toxemia. Quigley and Waite (1931) found the toxic dose to be about 0.2 per cent of the body weight and the minimum lethal dose 0.25 per cent of the body weight of fowls. Heuser and Schumacher (1941) reported that 5 per cent of the ration or 0.3 per cent of the body weight was toxic for chickens 6 to 10 weeks old. They found that a tolerance to the poison is frequently developed so that 0.4 to 0.5 per cent body weight could be consumed without materially affecting the growth. Ten per cent of the ration or 0.8 per cent body weight was lethal to some of their experimental fowls.

The clinical symptoms observed are a decided decrease in the respiration and heart rate, caseous lesions on the mucous membranes of the mouth, and diarrhea, the severity of which depends on the amount of toxic feed consumed. The lesions include yellow caseous exudate on the lining of the crop and varying degrees of gastroenteritis. Accumulations of clear amber fluid under the serosa of the digestive tract and in the pericardium, hemorrhages, and congested areas on the heart may be observed. Various degrees of congestion may be encountered together with degenerative changes in the liver.

Cottonseed meal. The active principle of cottonseed products which is toxic to both animals and birds is gossypol. Cottonseed meal has been used as a protein supplement in stock feed for many years, and if properly used in a mixed grain ration is a valuable and economic protein supplement. Excessive quantities, however, produce toxic effects often terminating in death. Kaupp (1933) studied the toxic properties of cottonseed meal for poultry. He concluded that toxic effects were soon observed in birds consuming 1 ounce of cottonseed meal daily or its equivalent in gossypol. Clinical symptoms of generalized toxemia appear with loss of appetite, cyanosis, and emaciation, followed by death in a few days after the appearance of the first clinical symptoms. The lesions include cyanotic appearance of the comb and wattles, varying degrees of gastroenteritis, and degenerative changes in the liver and kidneys.

Coyotillo. Losses in poultry by the consumption of the fruit and seed of the coyotillo plant (*Karwinskia humboldtiana*) have been reported. This plant is indigenous to southwestern Texas and Mexico. Marsh and associates (1928) reported that the clinical symptoms of this form of poisoning do not appear for several days and may appear as late as 3 weeks after the ingestion of the toxic substance. The toxic dose for chickens was found to be 0.3 per cent or more of the live weight, fed as dried fruit or seed. Symptoms of generalized toxemia appear, followed by progressive paralysis and death.

Crotalaria seed. The toxicity of certain species of crotalaria seeds for the chicken, quail, and dove was reported by Thomas (1934). Turkeys appeared to be more resistant to their toxins. However, crotalaria poisoning was not a serious problem until the introduction of mechanical pickers for corn and soybeans and the intensive use of crotalaria in the Southeast to increase the humus and nitrogen content of the soil (Kelley *et al.*, 1961; Smith and Osborne, 1962). Although there are hundreds of species of crotalaria, Smith and Osborne (1962) reported only a few are toxic for poultry. *Crotalaria spectabilis* appears to be the most toxic species for poultry, but *C. giant striata* was found toxic by Kelley *et al.* (1961), and Emmel (1937b) found *C.*
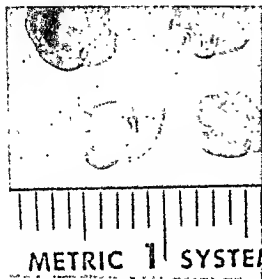
FIG. 40.15 – Crotalaria spectabilis seeds. Note smooth glassy seed coat and mitten-shaped appearance. ×4.

retusa toxic for chickens The small seeds are black or greenish brown with a smooth surface and have a characteristic mitten shape (Fig 40.15). A toxic alkaloid known as monocrotalin was extracted from Crotalaria spectabilis seed by Thomas et al. (1935). Under experimental conditions, Kelley et al. (1961) found that 1 per cent of C. spectabilis in the diet of day-old chicks killed all the chicks by 4 weeks of age. Studies by Bierer et al. (1960) showed that 0.2 of a pound of Crotalaria specta-

bilis seeds per ton of feed (0.01 per cent) was toxic for chickens.

Caylor (1961) found that 0.05 per cent Crotalaria spectabilis in the feed lowered production after 4 weeks and had a pronounced effect by 6 weeks. At a 0.1 per cent level, production practically ceased by 6 weeks. These results showing the effect of Crotalaria sp. on egg production were confirmed by Harms et al. (1963). They also observed that the inclusion of 64 Crotalaria spectabilis seeds per pound of feed for 9 weeks did not cause mortality in pullets.

Poisoning may occur in the acute or chronic form, terminating fatally in from 1 day to several months. Affected chicks become droopy, inactive, and have ruffled feathers. There is a tendency for the birds to huddle, and feed consumption decreases. Growth is retarded and the birds are stunted and inactive (Fig. 40.16). Birds with accumulations of abdominal fluid manifest a ducklike attitude when walking. In mature birds the comb and wattles are pale and egg production gradually drops with a corresponding increase in mortality.

The type and extent of the lesions vary with the duration of the condition and the age of the birds. Young birds may have subcutaneous edema and ascites. Hydropericardium may be present occasionally but is not a prominent lesion. Early in the course of the disease the liver is

FIG. 40.16 – Crotalaria spectabilis poisoning. Note stunting of the chick on the right compared with normal chick on the left.

FIG. 40.17 — Crotalaria spectabilis poisoning in a 2-week-old chick. The visible lobe of the liver is yellow and atrophied and the other lobe is covered by ascitic fluid.

swollen and mahogany colored but later becomes atrophied and cirrhotic (Fig. 40.17). Mature birds may die acutely from massive hemorrhage from the liver. Lesions described by Emmel (1937a) were numerous petechiae in the serous membranes and visceral fat (Fig. 40.18). The liver is

FIG. 40.18 — Hemorrhages of the epicardium and myocardium in acute Crotalaria spectabilis seed poisoning in a chicken. (Emmel, Jour. A.V.M.A.)

mottled and the kidneys show evidence of nephritis. Simpson et al. (1963) reported that the most prominent lesions in poults were focal or diffuse hemorrhages visible on the surface of the liver and extending into the parenchyma. Hemorrhages were also present on the epicardium and in the pectoral musculature.

Simpson et al. (1963) found at necropsy of pullets that had been fed Crotalaria-contaminated diets for nine weeks that the walls of the pericardial sac and air sacs were thickened. The lungs were edematous and the wall of the proventriculus was thickened. The livers were either dark and swollen or atrophied, knobby, and grayish tan. The spleens were swollen and pulpy.

Histopathological changes reported by Kelley et al. (1961) and Simpson et al. (1963) are most prominent in the liver. There is a fibrous thickening of the liver capsule and vacuolation or granular degeneration of the hepatic cells. Productive tissue changes are present in the region of the portal triad associated with bile duct hyperplasia and increased connective tissue. Occlusion of the small branches of the portal veins is caused by swollen endothelial cells and subendothelial edema. The kidneys may have interstitial edema,

dilatation of Bowman's space, and hyaline casts in some tubules. In the spleen there is a depletion of the splenic corpuscles and necrosis of persistent germinal centers. Occasionally there is a lymphocytic interstitial myocarditis and separation of myocardial fibers by edema. Hypoplasia of the bone marrow is found in chicks.

In young birds, the lesions produced by Crotalaria poisoning resemble those produced by "toxic fat," salt poisoning, cresol poisoning, and alimentary exudative diathesis. Tissues from affected birds and the suspected feed sample can be analyzed for the presence of monocrotalin, the toxic alkaloid in *Crotalaria spectabilis* seed. In acute cases of poisoning the presence of the characteristic mitten-shaped seeds in the crop or gizzard aid in making a diagnosis.

Daubentonia seeds. The Daubentonia (*Daubentonia longifolia*), also called the Sesbania, is a native of Mexico but was introduced to the southern states as an ornamental shrub. The seeds of this plant are readily eaten by poultry and are extremely toxic According to Shealy and Thomas (1928), the ingestion of as few as nine seeds will cause death in birds. The first clinical symptoms observed are a staggering gait, accompanied by drooping of the wings. Depression, general debility, and unthriftiness soon become apparent. The comb becomes cyanotic, and the head may hang over to one side Muscular twitching, diarrhea, emaciation, and extreme weakness are followed by death in 24 to 72 hours after the appearance of the first symptoms. The lesions include severe gastroenteritis with ulceration of the proventriculus and gizzard together with degenerative changes of the liver.

Death camas. The death camas belong to the genus Zygadenus, and the members of this genus, according to Marsh *et al.* (1915), generally conceded to be poisonous are *Z. glaberrimus, Z. intermedius, Z. mexicanus, Z. nuttallii, Z. paniculatus,* and *Z. venenosus.* Niemann (1928) reported an outbreak of poisoning in the domestic fowl due to the ingestion of *Z. nuttallii.*

This plant is not very palatable and is only eaten by fowls on the range in the early spring and late fall when other green feed is comparatively scarce. Experimental feedings of 5 to 10 grams to chickens produced marked clinical symptoms in 12 hours, including salivation, incoordination, muscular weakness, and diarrhea, followed by prostration and death. No definite lesions are associated with this type of poisoning. A strong, penetrating, disagreeable odor from the internal organs was noted, and muscular atrophy was observed. The mesenteric and abdominal blood vessels appeared congested. The lumen of the digestive tract was noticeably diminished in size.

Glottidium seed. *Glottidium vesicarium* (Jacq.) Harper is quite common along the coastal plain from North Carolina to Florida and Texas, having been introduced from the West Indies. The seeds of this plant are toxic for poultry. Under ordinary conditions fowls do not select these seeds as food but if underfed may consume sufficient quantities to be toxic. Emmel (1935) produced toxic effects by experimental feeding of *G. vesicarium* seeds to fowls. The clinical symptoms in acute poisoning are prostration, and cyanotic appearance of comb and wattles accompanied by diarrhea. In chronic cases the feathers become ruffled, copious yellow diarrhea persists, and the birds may become emaciated. The combs appear light in color and become scaly. The most characteristic lesions observed include necrotic enteritis, necrotic areas in the lining of the gizzard, and degenerative changes in the liver and kidneys.

Vetch seed. Reports of toxicity as the result of feeding large quantities of vetch seed to chickens are recorded in the literature. Little reliable information can be secured on the toxic principles of the vetches from the reports published either in this country or abroad. Numerous incidents of poisoning, commonly referred to as "lathyrism," are recorded in which birds show severe central nervous disturbances including spasmodic convul-

sions, paresis, and death following the use of large quantities of Lathyrus peas in the feed. Stockman (1931) regarded the nature of this toxic reaction highly controversial. Anderson and his associates (1925) indicated that the seed of *Lathyrus sativus* is not toxic unless contaminated by seeds of a variety of vetch, *Vicia sativa* L., var. *angustifolia*. Horvath (1915) reported that the vetchling (*Lathyrus cicera*), when fed as a sole food, seemed to exert a toxic effect on hens, resulting in a loss of weight. No characteristic pathologic lesions were produced. Harper and Arscott (1962) reported that the seed of common vetch, *Vicia sativa*, was toxic and lethal when fed at levels of 20 to 40 per cent in practical type rations to poults and chicks. At the 30 per cent level, 70 per cent mortality occurred in the poults and 100 per cent occurred in the chicks within one to four weeks after feeding the toxic mash. Autoclaving the ground vetch seed for 8 hours reduced toxicity and significantly improved growth. The seed of hairy vetch, *Vicia villosa*, was less toxic for poults and chicks. Several varieties of vetch seed contain a cyanogenic glucoside, "vicianin," which is decomposed by an enzyme (vicinase) into hydrocyanic acid, benzaldehyde, and a disaccharide (vicianose). It is possible that large quantities of such feed could cause considerable loss in a flock of birds.

Milkweed. Two of our common species of milkweed, *Asclepias tuberosa* and *A. incarnata*, contain the bitter glucoside asclepidin, which apparently is toxic to animals and birds. Pammel (1911) includes *A. vestita* and *A. mexicana* in the list of toxic species of the milkweed family. Campbell (1931) reported serious losses in poultry caused by the consumption of the narrow-leaved, whorled milkweed, *A. mexicana*. Experimental investigations indicated that all parts of the plant are toxic. Pammel (1917) reported milkweed poisoning in chickens resulting in the loss of approximately 500 birds. Stiles (1912) reported extensive losses in turkey poults resulting from the consumption of whorled milkweed (*Asclepias gale-*

ordes). Poultry, as well as other species of livestock, are not likely to eat milkweed except when it is the only succulent feed available.

The clinical symptoms may vary considerably, depending on the quantity of the toxic material eaten. The first symptom observed was lameness which developed rapidly into complete loss of muscular control. The neck became twisted and the head drawn back. The affected birds often lay on their sternums or sat on their hocks alternately extending and retracting their heads at frequent intervals. This condition did not seem to be a true paralysis but appeared to be an overstimulation of the motor nerve centers with complete loss of coordination. At times the birds would fall over and struggle, with violent convulsive movements of the legs. In some instances the symptoms gradually subsided, followed by recovery. In fatal cases the symptoms became progressively worse, followed by prostration, coma, and death. No characteristic lesions were found upon necropsy.

Nightshade. The black nightshade (*Solanum nigrum*) is a common weed in yards and poor pasture land. The immature fruit of this plant contains the alkaloids solanin and solanidin which are toxic to man and animals. The toxicity of the plants is believed to be influenced by the soil, climate, and degree of plant maturity. Hansen (1925) reported fatal poisoning in chickens and ducks attributed to black nightshade. The clinical symptoms include incoordination, prostration, paralysis, and death. The pupils of the eyes may be dilated. No characteristic lesions are reported except evidence of severe toxemia.

Lily of the valley and oleander. The flowers, leaves, and stems of the lily of the valley (*Convallaria majalis*) and the leaves of the oleander (*Nerium oleander*) were reported by Bartosi (1939) to be poisonous for geese, ducks, and hens. The lily of the valley flowers were found to be lethal to geese in doses of 15 grams and to ducks in doses of 12 grams. Doses of 50

grams produced only a mild enteritis in mature chickens.

The dried leaves of the oleander plant of the previous season's growth proved fatal to geese in 24 hours after the ingestion of 6 grams. The lethal dose for ducks was found to be 3 grams. The young leaves of this plant proved fatal to hens in doses of 15 grams. Hinshaw reported losses in turkey poults within 24 hours as the result of eating young shoots of oleander. The postmortem examination of the poults showed hemorrhagic enteritis. (See chapter on Diseases of the Turkey.)

The clinical symptoms of oleander poisoning include general depression, weakness, diarrhea, accelerated heart rate, impaired vision, muscular incoordination, and in some instances paralysis of the wings. Various degrees of gastroenteritis and liver degeneration occur in fatal cases.

Potatoes. Under certain conditions the potato (Solanum tuberosum) is poisonous to domesticated animals and poultry. Greened tubers, produced by exposure to light, and young potato sprouts contain considerable amounts of the alkaloid solanin which is highly toxic Analyses have shown that the solanin content of the sprouts and peelings is higher than that of the interior of the tuber. Hansen (1927) reported several outbreaks of poisoning in poultry resulting from the ingestion of potato sprouts. Losses occurred within a few hours after the consumption of the toxic sprouts. Temperton (1944) reported losses in ducks as the result of eating either cooked or uncooked sprouted potatoes. This type of poisoning is similar to that of poisoning by other plants of the nightshade family. However, where large amounts of potatoes are fed to poultry, it is advisable as a safety measure to cook green or sprouted potatoes and to discard the residual water. From the standpoint of nutrition, raw potato starch is poorly digested by poultry, and cooking will result in a more efficient utilization of this type of feed.

Tobacco. The tobacco plant (Nicotiana

tobacum L.) contains the toxic alkaloid nicotine. Hunter and associates (1931, 1934) reported that growing chicks over 3 weeks of age can tolerate as much as 0.06 per cent nicotine in the ration without any toxic reaction. The feeding of the same nicotine levels in the form of ground cigar clippings having only 0.86 per cent nicotine content retarded the growth and development of chicks, causing some losses. Toxic doses of tobacco produce similar reactions to those of nicotine sulfate poisoning.

Algae. Certain types of algae, including the Microcystis aeruginosa, which grow abundantly in lakes under certain conditions, may become concentrated in localized areas by the action of strong winds blowing the surface of the water in one direction for a number of days. Great quantities of algae are deposited on the banks and in the shallow waters along the shore line. The disintegration of this material produces toxins which are responsible for the loss of various species of wildlife as well as domesticated animals and birds. This condition has frequently been called "water bloom." Fitch and his associates (1929) reported losses of livestock from this cause in Minnesota. Brandenburg and Shigley (1947) reported this condition in North Dakota. It has also been reported in northern Iowa and in parts of Canada. It usually occurs in the latter part of July, August, and September. The exact nature of the toxin is not known at the present time. Apparently, the toxin is an intermediate product of disintegration, as these toxic properties disappear during the latter stages of decomposition.

The toxicity of this material is directly proportional to the concentration. Some waters taken from the shores of lakes are extremely toxic to birds and animals. Under experimental conditions, oral dosages of 10 cc. to 30 cc. will produce death in mature ducks and chickens in 10 to 45 minutes. The toxin is thermostable and is affected little, if any, by boiling. The

symptoms include restlessness, twitching of muscles, nervous manifestations, spasms, convulsions, paralysis, and death. These clinical manifestations resemble strychnine poisoning in many respects. The postmortem lesions include generalized cyanosis; dark, tar-colored blood; liver congested and dark in color; heart dilated and distended; muscles also congested and dark in color. No characteristic hemorrhages are observed. Ashworth and Mason (1946) described in detail the symptoms and lesions associated with algae poisoning in laboratory animals.

Because of the highly toxic nature of this material and the rapidity with which it acts, there are no therapeutic measures effective. Poultry should be restricted to areas free from toxic material.

Nontoxic algae may be responsible for considerable losses in young chickens. The mature birds are seldom affected, but the young chicks which wade out along the shore line and feed on insects alighting on the floating scum or masses of algae, invariably swallow various amounts of this material. This scum becomes lodged in the nostrils as well as the digestive and respiratory passages, causing strangulation and suffocation. Symptoms characteristic of strangulation are observed. Postmortem examination reveals obstruction of the nostrils and respiratory passages as well as those of the digestive tract.

Losses of poultry associated with the ingestion of algae should be investigated at once. The toxicity of algae can be determined readily by injecting 2 cc. or more of a sample filtered through gauze, intraperitoneally into laboratory animals or chickens. Toxic material will produce typical clinical symptoms in a short time and death within an hour or so. A prompt determination of toxicity may prevent serious losses to all species of livestock.

INSECTS

Insects of various kinds annoy poultry and act as intermediate hosts for poultry parasites, but only a few of those eaten by birds are considered poisonous. Only one insect is described which, if eaten in sufficient numbers, produces a severe toxic reaction in chickens, i.e., the rose chafer.

Rose chafer. Rose chafers (*Macrodactylus subspinosus*) are abundant during the latter part of May and June, and early July in Canada and the eastern United States extending as far west as Colorado. The toxic properties of this insect for chickens have been reported by Bates (1916), Gallagher (1920), and Lamson (1916, 1922). Fatal cases of poisoning in young chickens of various ages have been recorded, but mature fowls are seldom killed. Chickens will feed ravenously upon these insects if available, and 15 to 20 rose chafers are sufficient to kill a chicken 1 week old, while birds about 3 weeks of age show a toxic reaction after eating 25 to 45 of these insects. A fatal reaction usually results in 21 hours, or the birds gradually recover. Watery extracts made from crushed rose chafers proved toxic when administered to chickens. Lamson was of the opinion that the poisonous principle is a neurotoxin which has a direct effect on the heart action.

The clinical symptoms include drowsiness, incoordination, weakness, prostration, convulsions, and retraction of head and neck over the back of the affected chicken. Death usually occurs in less than 21 hours subsequent to eating rose chafers or from ½ to 1 hour after the appearance of the first symptoms. The postmortem examinations fail to show characteristic lesions other than injection of the blood vessels of the heart in some cases.

MISCELLANEOUS FOOD POISONS

Selenium. Toxic reactions from eating grains grown in certain limited areas, due to specific toxic mineral constituents, have been reported by Franke and associates (1931) in parts of South Dakota. The so-called alkali disease was found to be due to the high selenium content of the grain grown in that locality. Losses of livestock from selenium poisoning were reported

by Moxon (1937). Studies at the South Dakota Experiment Station indicated that toxic grains fed to laying hens at levels which contained 15 p.p.m. of selenium resulted in reduced weight, caused a decided reduction in egg size, and practically destroyed the hatchability. According to Poley and associates (1937), the feeding of 5 p.p.m. of selenium did not appreciably affect the hatchability even though some evidence of selenium poisoning was apparent.

Toxic fat syndrome. During the fall of 1957, severe outbreaks of a new disease syndrome characterized by ascites, hydropericardium, and subcutaneous edema occurred in broilers in the central and southeastern United States. Schmittle et al. (1958) and Sanger et al. (1958) reported on clinical observations and laboratory findings. They determined that certain samples of fat or feed containing this fat would produce the edematous condition when fed to chickens. Wannop and Chubb (1961) in Great Britain reported a syndrome characterized by ascites and hydropericardium in broilers. They indicated that fat had been added to the feed. Alexander et al. (1962) reported that the source of the toxic factor was traced to specific lots of feed grade animal fats containing a residue from some fat-processing operations. They further indicated that it was believed that the residue contained either a contaminant, or, in the course of the fat processing, a new compound was formed which was toxic for chicks. Investigation by Friedman (1962) indicated that some vegetable fat sources may yield fatty acids contaminated with the chick edema factor.

Signs. Clinical signs may appear as early as 3 weeks of age and are manifested by dyspnea, ruffled feathers, droopiness, paleness, stunting, and sudden death. Some of the chicks may have a waddling, unsteady gait or a penguinlike posture. Blood counts reveal a marked anemia, and Simpson et al. (1959) reported erythrocyte counts as low as 800,000 per cubic millimeter. In blood smears, immature erythrocytes can be

seen. Mortality as high as 90 per cent has been reported.

Lesions. Birds in the advanced stages of the disease have large, fluctuating, distended abdomens containing clear straw-colored fluid with large fibrinous clots (Fig. 40.19). Subcutaneous edema occurs in the region of the breast, abdomen, and thighs. The most consistent lesion is a greatly distended pericardial sac filled with amber-colored fluid (Fig. 40.20). The heart is slightly enlarged and the myocardium is pale. Occasionally, petechiae may be present in the myocardium. Hemorrhages may occur in the skeletal muscles but are not a constant feature of the disease. The crop contents may be bloody and the buccal cavity, beak, and head region may be blood stained. The lesions in the liver are variable depending upon the duration of the condition. A removable layer of yellow coagulated serum may cover the surface (Fig. 40.21). In the early stages the liver is enlarged and mottled with irregular, diffuse, lighter-colored areas resembling fatty change interspersed with red streaks or patches. In chronic cases, the liver is shrunken, nodular, firm, and has a nut-

FIG. 40.19 — Toxic fat syndrome. Severe ascites in a 7-week-old chicken.

FIG. 40.20 — Toxic fat syndrome. Marked distension of the pericardial sac with fluid.

meg or bronze color. The kidneys are pale, swollen, with occasional hemorrhage occurring beneath the capsule of the anterior lobes.

Effect on pullets and layers. Dunahoo *et al.* (1959) studied the effect of toxic fat in the rations of laying hens and pullets. Pullets receiving a diet containing 5 per cent toxic fat from the twelfth to the fourteenth week of age came into production two weeks later than the controls.

Their rate of production was 20 per cent below that of the controls and hatchability was decreased. Pullets receiving 5 per cent toxic fat in the ration for 61 days did not come into production. The growth rate of these pullets was depressed by feeding toxic fat. Sixty-seven per cent of the birds receiving toxic fat for the full growing period died during the course of the experiment. Birds that died before laying age frequently had hydropericardium,

FIG. 40.21 — Toxic fat syndrome. Ascitic fluid in peritoneal cavity and a fibrinous layer over the surface of the liver.

whereas this lesion was rarely present in birds of laying age. Other lesions were ascites, enlarged and ruptured livers, and pale, swollen kidneys. Laying birds fed 5 per cent toxic fat in the ration practically ceased production in 2 weeks time. The hatchability of eggs from birds fed toxic fat was very low. Production was only slightly improved 6 weeks after the feeding of the toxic fat had been discontinued.

Effect on cockerels. Allen and Lalich (1962) reported testicular hypoplasia in cockerels following prolonged feeding of toxic fat at a level of 0.25 per cent in the diet. The rate of growth was not appreciably affected when this concentration of toxic fat was fed. Despite the testicular hypoplasia, the manifestation of such secondary sex characters as comb size and body conformation was not delayed. Toxic fat at levels of 0.5 and 1.0 per cent in the feed caused testicular hypoplasia in conjunction with ascites and hydropericardium. Whereas, when the level of toxic fat was reduced to 0.25 per cent, ascites and hydropericardium were markedly reduced but retarded testicular development was found consistently.

Other species. Simpson et al. (1959) indicated that clinical cases occurred in turkeys as well as chickens but Sanger et al. (1958) stated that according to all reports the disease was confined to chickens. Edgar et al. (1958) fed 4 per cent toxic fat to ducks and turkeys for 6 and 11 weeks respectively but failed to produce any pronounced signs or lesions of the disease.

Histopathology. Simpson et al. (1959) and Sanger et al. (1958) described the histological changes found in the toxic fat syndrome. Liver lesions consisted of areas of focal necrosis, cellular degeneration, and numerous petechiae. Bile duct proliferation was evident and there were accumulations of crystallized bile salts in the bile ducts. In advanced cases there was destruction of nearly all the liver cells which was accompanied by fibrosis and infiltration of heterophiles and lymphocytes. Heart lesions were myocardial degeneration and separation of the muscle fibers, with focal

hemorrhages, and lymphocytic infiltration. The kidneys had interstitial edema and enlarged glomeruli that were bloodless due to the proliferation and swelling of the endothelial cells. Vascular lesions in most tissues were an endotheliosis consisting of proliferation and hypertrophy of the endothelial cells of arterioles. Hyaline casts and fibrin were present in some kidney tubules.

Studies on the toxic factors. Ott et al. (1961) described a chick assay procedure for the edema-producing factor in toxic fat. Detectable hydropericardium was produced by feeding 7 parts of pure edema-producing factor per billion parts of diet in a 20-day feeding period. Mortality was caused when 64 or more parts of the pure factor were present per billion parts of diet. On the basis of comparative assay results it was concluded that the concentration of the pure factor in the toxic fat standard was approximately 0.5 p.p.m. After a 21-month holding period, the toxic fat was calculated to be 72 per cent as toxic as it was initially. This comparatively small loss in the activity of the chick edema factor indicates it is very stable in toxic fat.

Wootton et al. (1962) reported the isolation of three hydropericardium-producing factors from a toxic fat. Two of the compounds were isolated in a pure form. Investigation indicated they possessed a high melting point and high molecular weight. It was also determined that they were crystalline compounds containing six chlorine atoms per mole and appeared to have an aromatic nucleus.

Diagnosis. In making a differential diagnosis of the toxic fat syndrome it would be necessary to eliminate the possibility of cresol poisoning, salt poisoning, crotalaria poisoning, and exudative diathesis.

Cresol poisoning is only of sporadic occurrence in an occasional flock. A careful history of the case would reveal that a cresol disinfectant had been used prior to housing the flock.

Salt poisoning caused by excessive amounts of NaCl in the feed or water

could be detected by analysis of these dietary components for their salt content. Sanger *et al.* (1958) indicated that the extreme liver necrosis found in the toxic fat syndrome was not found in salt poisoning.

Crotalaria poisoning would be more likely to occur in those regions where crotalaria is grown and therefore would tend to be regional in occurrence. Feed suspected of containing *Crotalaria spectabilis* could be analyzed for the toxic alkaloid monocrotalin.

The paucity of reports describing naturally occurring cases of exudative diathesis would indicate that this condition is rarely seen in the field. The marked liver changes found in cases of toxic fat poisoning have not been described for vitamin E deficiency. In field cases of exudative diathesis, Thompson and Smith (1953) described muscular dystrophy of the skeletal muscles and myopathy of the gizzard muscle. In addition, microscopic examination of the brain revealed cerebral edema and hemorrhage. Ascites and marked hydropericardium, a constant feature of the toxic fat syndrome, are not mentioned as prominent changes in exudative diathesis. Controlled feeding trials using supplementary vitamin E would determine whether this vitamin was involved in the disease in question.

If the conditions resembling toxic fat syndrome can be eliminated, then a presumptive diagnosis can be made on the basis of history, signs, and gross and microscopic lesions. If fat still remained from the lot that was used in the suspect feed a bioassay determination could be performed by feeding this fat to day-old chicks. The development of hydropericardium and ascites following the feeding of the suspect fat would indicate its toxicity.

Protein poisoning. Protein poisoning in poultry may be both quantitative and qualitative. Jull (1930) points out the harmful effects of excessive protein in the ration. The clinical symptoms caused by excessive amounts of protein are those of generalized toxemia including depression, leg weakness, prostration, and coma, followed by death. This problem is primarily one of management, and losses from this cause can be prevented by using properly balanced rations.

Birds may also be poisoned by the ingestion of proteins of poor quality such as partly decomposed foods. Decomposed proteins frequently prove toxic if fed in sufficient amounts. It is believed that the products of disintegration are responsible for the toxic reaction, which is not necessarily associated with bacterial toxins produced as in the case of botulism.

Feeds of various kinds, whether they are proprietary feed mixtures or some ingredient of home mixed rations, are often suspected of being responsible for poultry losses. Quigley and Waite (1931) reported that investigation on numerous samples examined over a period of 3 years failed to show any toxic reaction in actual feeding trials. Various other reports indicated that comparatively few feeds suspected of being poisonous proved to be so when examined.

The losses in poultry as the result of poisoning from any cause are exceedingly small compared to the losses attributed to infectious diseases, parasites, and other causes. Positive diagnosis of poisoning depend entirely upon the discovery of the poison in the bird by chemical analyses or by the detection of a specific poison in the food supply.

Allen, J. R., and Lalich, J. J.: 1962. Response of chickens to prolonged feeding of crude "toxic fat." Proc. Soc. Exper. Biol and Med 109 48

Anderson, L. A. P., Howard, A., and Simonsen, J. L.: 1925. Studies on lathyrism. Indian Jour. Med. Res. 12:613.

Anderson, W. A., and Richter, C. P.: 1946. Toxicity of alpha naphthyl thiourea. Vet. Med. 41:302.

Archibald, R. McG., Smith, H. J., and Liddle: D.: 1962. Brazilian groundnut toxicosis in Canadian broiler chickens. Canad. Vet. Jour. 3:322.

Ashworth, C. T., and Mason, M. F.: 1946. Observations on the pathological changes produced by a toxic substance present in blue green algae (Microcystis aeruginosa). Am. Jour. Path. 22:369.

Asplin, F. D., Carnaghan, R. B. A.: 1961. The toxicity of certain groundnut meals for poultry with special reference to their effect on ducklings and chickens. Vet. Rec. 73:1215.

Barber, P. G., and Hubster, E. B.: 1933. Arsenic poisoning in poultry. No. Am. Vet. 28:500.

Bardosi, Z.: 1939. Toxicity of lily of the valley and oleander leaves for fowls (trans. title). Thesis, Budapest, Abst. Vet. Bul. (1940) 10.624.

Barlow, J. S., Slinger, S. J., and Sumner, R. P.: 1948. The reaction of growing chicks to diets varying in sodium chloride content. Poultry Sci. 27:542.

Barnes, M. F.: 1921. Black locust poisoning of chickens. Jour. Am. Vet. Med. Assn. 59:370.

Bates, J. M.: 1916. The poisonous character of rose chafers. Science 43.209.

Beach, J. R.: 1950. Intestinal worms of poultry. No. Am. Vet. 11:45 (Nov).

Beaudette, F. R., Hudson, C. B., and Weber, A. L.: 1933. Phosphorous poisoning in poultry. No. Am. Vet. 14:39 (July).

Bierer, B. W.: 1958. The ill effects of excessive formaldehyde fumigation on turkey poults. Jour. Am Vet. Med Assn 132 174

——, Richter, C. F., Roebuck, D. E: 1963. Effect of ingested disinfectants on chicks. Jour. Am. Vet. Med. Assn 142 512

——, Vickers, C. L., Rhodes, W. H., and Thomas, J. B.: 1960. Comparison of the toxic effects of Crotalaria spectabilis and Crotalaria giant striata as complete feed contaminants. Jour. Am. Vet. Med. Assn 136.318.

Bigland, C. H 1950 Ascites and edema of brooded turkey poults in Alberta, Canada. Canad. Jour. Comp. Med. 14:144

Blaxland, J. D.: 1946. The toxicity of sodium chloride for fowls. Vet. Jour. 102:157.

Bleecker, W. L., and Smith, R. M.: 1933a. Further studies on the relative efficiency of vermifuges for poultry. Jour. Am. Vet. Med. Assn. 83-76

——, and Smith, R. M.: 1933b. Nicotine sulfate as a vermifuge for the removal of ascarids from poultry. Jour. Am. Vet. Med. Assn. 83:645.

Blount, W. P.: 1961 Turkey "X" disease. Jour, Brit. Turkey Fed. 9:52.

Bootes, D. W.: 1962 Poisoning of turkey poults with lindane. Aust. Vet. Jour. 38.67.

Brandenburg, T. O., and Shigley, F. M.: 1947. "Water Bloom" as a cause of poisoning in livestock in North Dakota. Jour. Am. Vet. Med. Assn. 110:384.

Bressler, G. O., Goetkeh, S. J., and Pritham, G. H.: 1951. The effect of salt and carbolineum in producing ascites in turkey poults. Poultry Sci. 30:738.

Buckley, J. S., Bunyea, H., and Cram, E. B.: 1939. Diseases and parasites of poultry. U.S.D.A. Farmer's Bul. 1052

Bullis, K. L., and Van Roekel, H.: 1944 Uncommon pathological conditions in chickens and turkeys. Cornell Vet 34:313.

Campbell, H. W.: 1931. Poisoning in chickens with whorled milkweed. Jour. Am. Vet. Med. Assn. 79.102

Carnaghan, R. B. A., and Blaxland, J. D.: 1957. The toxic effect of certain seed dressings on wild and game birds. Vet. Rec 69-324.

——, and Sargeant, K.: 1961 The toxicity of certain groundnut meals to poultry. Vet. Rec. 73:726.

Carpenter, C. P: 1931. The use of nicotine and its compounds for the control of poultry parasites. Jour. Am. Vet. Med. Assn 78 651.

Caylor, J. F, and Laurent, C. K.: 1961 Effect of level of Crotalaria spectabilis on egg production of White Leghorn hens. Poultry Sci. 40.818.

Coburn, D. R., Metzler, D. W., and Treichleer, R. R: 1951. A study of absorption and retention of lead in wild waterfowl in relation to clinical evidence of lead poisoning. Jour. Wildlife Mgt. 15:186.

Cooley, R. A., Parker, J. R., and Strand, A. L.: 1923. Improved methods of controlling grasshoppers. Mont Agr. Exper. Sta. Circ 112.

Costigan, S. M.: 1910. Lead poisoning in guinea fowl. Jour. Am. Vet. Med. Assn. 97:451.

Cottral, G. E., Dubble, G. D., and Winton, B.: 1947. The effect of sodium fluoroacetate ("1080" rodenticide) on White Leghorn chickens. Poultry Sci. 26.610.

Cram, E. B.: 1928. The present status of our knowledge of poultry parasitism. No. Am. Vet. World's Poultry Congress.

Davies, S. F. M.: 1954 Sulfonamide poisoning in chickens treated for coccidiosis. Proc. Tenth World's Poultry Congress.

———, and Kendall, S. B.: 1953. Toxicity of sulfaquinoxaline for chickens. Vet. Rec. 65:85.

Delaplane, J. P.: 1934. Some of the tissue changes in poultry resulting from the ingestion of sodium bicarbonate. Vet. Alumni Quart. (Ohio State Univ.) 21:149

———, and Milliff, J. H.: 1948. The gross and micropathology of sulfaquinoxaline poisoning in chickens. Am. Jour. Vet. Res. 9:92.

Doll, E. R., Hull, F. E., and Insko, W. M.: 1946. Toxicity of sodium chloride for baby chicks Vet. Med. 41:361.

Dougherty, E., III: 1957. Thiophosphate poisoning in white Pekin ducks. Avian Dis. 1.127.

Dumahoo, W. S., Edwards, H. M., Jr., Schmittle, S. C., and Fuller, H. L: 1959. Studies on toxic fat in the rations of laying hens and pullets. Poultry Sci. 38 663.

Edgar, S. A., Bond, D. S., Melius, P., and Ingram, C. R.: 1958. The effect of a toxic substance in fat on poultry. Poultry Sci. 37:1200.

Edwards, J. T.: 1918. Salt poisoning in pigs and poultry. Jour. Comp. Path. and Therap. 31:40

Emmel, M. W: 1935. The toxicity of Glottidium vescarum (Jacq.) Harper seeds for the fowl Jour. Am. Vet. Med. Assn. 87:13.

———: 1937a. The pathology of Crotalaria spectabilis Roth seed poisoning in the domestic fowl Jour. Am. Vet. Med. Assn. 90.627.

———: 1937b. The toxicity of Crotalaria retusa L. seeds for the domestic fowl Jour. Am. Vet. Med. Assn. 91:205.

Ewing, W. R.: 1947. Poultry Nutrition, 3rd ed. P 1047.

Farr, M. M., and Jaquette, D. S.: 1947. The toxicity of sulfamerazine to chickens. Am Jour Vet. Res 8:216.

———, and Wehr, E. E.: 1945. Sulfamerazine therapy in experimental cecal coccidiosis of chickens. Am. Jour. Parasitol. 31:353.

Fitch, C. P., Bishop, L., and Boyd, W. L.: 1929. "Water Bloom" as a cause of poisoning in domestic animals. Cornell Vet. 24:30.

Flick, D. F., Winbush, J., and Friedman, L.: 1962. Bioassay of chick edema factor. Food and Drug Administration. Washington 25, D.C.

Franke, K. W., Rice, T. D., Johnson, A. C., and Schoening, H. W: 1934 Report on a preliminary field survey of the so-called "alkali disease" of livestock. U.S.D.A., Circ. 320.

Friedman, L.: 1962. Progress in the chick edema problem Feedstuffs. 34:18.

Gaafar, S. M., and Turk, R. D.: 1957. The toxicity of malathion in chickens. Am. Jour. Vet Res. 18:180.

Gallagher, B. A.: 1919. Experiments in avian toxicology. Jour. Am. Vet. Med. Assn. 54:337.

———: 1920. Rose-chafer poisoning in chickens. Jour. Am. Vet. Med. Assn. 57.692

———: 1924. Canned goods preserved with boric acid poisonous to chickens. No Am. Vet 5:125.

Gibson, E. A.: 1957. An outbreak of sodium chloride poisoning in turkey poults. Vet. Rec. 69:1115.

Glover, J. S.: 1932. Mercurial poisoning in fowl. Rept. Ontario Vet. Coll 1931:56.

Golz, H. H., and Shaffer, C. B.: 1955. Malathion, summary of pharmacology and toxicology Am. Cyanamid Co., N.Y., Tech. Bul.

Gordon, R. S, Mulholland, R. A., Machin, L. J., and Naddy, K. H.: 1959. Hydropericardium and ascites caused by excess salt and a factor in blood meal. Poultry Sci. 38:1209.

Guberlet, J. E.: 1922. Potassium nitrate poisoning in chickens with a note on its toxicity. Jour. Am. Vet. Med. Assn. 62:362.

Hall, M. C., and Shillinger, J. E: 1926. Kamala, a satisfactory anthelmintic for tapeworms in poultry. No. Am. Vet. 7:51 (March).

Hansen, A. A.: 1925. Nightshade poisoning in chickens and ducks. Jour. Am. Vet. Med. Assn. 66.502.

———: 1927. Stock poisoning by plants in the nightshade family. Jour. Am. Vet. Med. Assn. 71:221.

Handzik, P. J., and Presho, E.: 1925. Comparative toxicity of inorganic lead compounds and metallic lead for pigeons. Jour. Pharmacol and Exper. Therap. 21:123.

Hare, T., and Orr, A. B: 1945. Poultry poisoned by zinc phosphide. Vet. Record 57:17.

Harman, R. E., Davis, G. E., Orr, W. H., Brink, N. G., and Kuehl, F. A., Jr.: 1960. The isolation and characterization of the chick edema factor. Jour. Am. Chem. Soc. 82:2078.

Harms, R. H., Waldroup, P. W., and Simpson, C. F.: 1963. Effect of feeding various levels of Crotalaria spectabilis seeds on the performance of chicks, turkeys, and pullets. Jour. Am. Vet. Med. Assn. 142:260.

Harper, J. A., and Arscott, G. H.: 1962. Toxicity of common and hairy vetch sred for poults and chicks. Poultry Sci. 41:1968.

Hawn, M. C.: 1935. The value of kamala as a tenicide for young turkeys. Jour. Am. Vet. Med Assn. 85:400.

Heinekamp, W. J. R.: 1925. The resistance of fowl to strychnine. Jour. Lab. and Clin. Med H:209.

Heuser, G. F., and Schumacher, A. E.: 1941. The feeding of corn cockle to chickens. Poultry Sci. 20:463.

Heuser, G. F., and Schumacher, A. E.: 1956. Feeding chemically treated seed grains to hens. Poultry Sci. 35:160.

Horvath, A. A.: 1945. Toxicity of vetch seed for chickens. Poultry Sci. 24:291.

Hudson, C. B.: 1936. Naphthalene poisoning in poultry. Jour. Am. Vet. Med. Assn. 89,219.

Hunter, J. E., and Haley, D. E.: 1931. The effect of various concentrations of nicotine in tobacco on the growth and development of fowls. I. A study of the nicotine tolerance of growing chicks. Poultry Sci. 10.61.

―――― Haley, D. E., and Knaidell, H. C.: 1934. Effect of concentrations of nicotine on growth and development. II. Growth and development of chicks as influenced by the addition of ground tobacco to the ration. Poultry Sci. 13 91.

Johns, F. M.: 1934. A study of punctate stippling as found in the lead poisoning of wild ducks. Jour. Lab. and Clin. Med. 19:514.

Johnson, E. L., Wahle, P. E., and Pomeroy, B. S.: 1955. The toxicity of Arasan-treated corn to hens and chicks Proc. Book, Am Vet. Med. Assn, 92nd Ann, Meet., p. 322.

Jones, J C.: 1939 On the occurrence of lead shot in stomachs of North American guiformes. Jour Wildlife Mgt 3 353.

Joyner, L. P., and Davies, S F M.: 1956. Sulphaquinoxaline poisoning in chickens. Jour. Comp Path. and Therap 66.39.

Jull, M A.: 1930 Poultry Husbandry. McGraw-Hill Book Co., Inc., New York. P. 328.

Jungherr, E.: 1935 Diseases of brooder chicks Storrs Agr. Exper. Sta. Bull. #202, Conn. State College, Storrs, Conn.

Kare, M. R., and Biely, J.: 1948 The toxicity of sodium chloride and its relation to water intake in baby chicks Poultry Sci 27:751.

Kaupp, B. F.: 1933 Poultry Diseases Sixth ed. Alexander Eger, Chicago, Ill. P. 444.

Kelles, J. W., Barber, C. W., Pate, D. D., and Hill, C. H. 1961 Effect of feeding crotalaria seed to young chickens. Jour Am. Vet Med. Assn 159:1215.

Kingscote, A. A., and Jarvis, G. H.: 1946. Report on experiments conducted to establish the tolerance of turkeys to DDT. Canad. Jour. Comp. Med 10 211.

Krakower, C. A., and Goettsch, M.: 1945. Effect of excessive ingestion of sodium chloride on the chick, with particular reference to renal changes. Arch. Path. 40:209.

Lamson, G. H., Jr.: 1916. The poisonous effects of the rose chafer upon chicks. Science 43:158 110.115.

―――― 1922. The rose chafer as a cause of death of chickens Storrs Agr. Exper. Sta. Bull 110.115.

Lander, G. D.: 1926a Veterinary Toxicology. Alexander Eger, Chicago, Ill. P. 57.

―――― 1926b. Veterinary Toxicology. Alexander Eger, Chicago, Ill. P. 75.

Levine, P. P.: 1939 The effect of sulfanilamide on the course of experimental avian coccidiosis. Cornell Vet. 29:309.

―――― and Barber, C W.: 1947. The comparative efficiency of some coccidiostatic agents against experimental infection with Eimeria tenella. Cornell Vet. 37:201.

McCune, E. L., Savage, J. E., and O'Dell B. L.: 1962 Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Poultry Sci. 41:295.

McNeil, E., and Hinshaw, W. R.: 1945. Effects of mercuric chloride on turkeys and on Hexamita meleagridis Poultry Sci. 24 516

―――― and Hinshaw, W. R.: 1947. Experience with DDT on a turkey ranch. Vet. Med. 42:181.

Machlin, L. J., Gordon, R S, Meinky, K. A., and Maddy, R. H.: 1959. Relationship of oxidative degradation to toxicity in certain fats. Poultry Sci. 38.579.

Marsh, C. D., Clawson, A. B. and Marsh, H.: 1915. Zygadenus or death camas. U.S.D.A. Bull. 125.

―――― Clawson, A. B. and Roe, G C.: 1928. Coyotillo (Karwinskia humboldtiana) as a poisonous plant. U.S.D.A. Tech. Bul. 29.

Marthedal, H. E., and Velling, G.: 1961. Hemorrhagic syndrome in poultry, British Vet. Jour. 177:357.

Morris, D. and Schmittle, S. C.: 1958. Toxic fat disorder of chicks. Proc. Poult. Path. Conf. Stony Point, N.Y.

Moxon, A. L.: 1937. Alkali disease or selenium poisoning. S. Dak. Agr. Exper. Sta., Bull. 311.

Neal, W. M., Rusoff, L. L., and Ahmann, C. F.: 1935. The isolation and some properties of an alkaloid from Crotalaria spectabilis Roth. Jour. Am. Chem. Soc. 57:2560.

Niemann, K. W.: 1928. Report of an outbreak of poisoning in the domesticated fowl, due to death camas. Jour. Am. Vet. Med. Assn. 73:627.

Nunn, J. A.: 1920. Veterinary Toxicology. Baillière, Tindall, and Cox, London. P. 2.

Orr, W. H., Dickinson, A. M., and Van Iderstine, A.: 1961. A chick assay procedure for the edema-producing factor in toxic fat. Poultry Sci. 40.169.

Pammel, L. H.: 1911. A Manual of Poisonous Plants. The Torch Press, Cedar Rapids, Iowa. P. 696

―――― 1917. Edematoid poisonous to chickens. Am. Jour. Vet. Med. 12:256.

Parker, S. L.: 1929. Effects of easily handicaps on chickens as measured by yolk absorption and body weight to twenty weeks of age. Hilgardia 4:1.

Paver, H., Robertson, A., and Wilson, J. E.: 1953. Observations on the toxicity of salt for young chickens. Jour. Comp. Path. 63:51.

Piercy, P. L., and Rusoff, L. L.: 1946. *Crotalaria spectabilis* poisoning in Louisiana livestock. Jour. Am. Vet. Med. Assn. 108:69.

Foley, W. E., Moxon, A. L., and Franke, K. W.: 1937. Further studies of the effects of selenium poisoning on hatchability. Poultry Sci. 16:219.

Post, G.: 1951. Effects of toxaphene and chlordane on certain game birds. Jour Wildlife Mgt. 15:381.

Pullar, E. M.: 1940a. The toxicity of various copper compounds and mixtures for domesticated birds. Australian Vet. Jour. 16:147.

————: 1940b. The toxicity of various copper compounds and mixtures for domesticated birds 2. Australian Vet. Jour. 16:203.

Quigley, G. D., and Waite, R. H.: 1931. Miscellaneous feeding trials with poultry. Md. Agr. Exper. Sta., Bul. 325:345.

————, and Waite, R. H.: 1932. Salt tolerance of baby chicks. Md Agr Exper. Sta., Bul 340 345.

Rae, R., and Crisp, C. S.: 1954. Lead poisoning in domestic ducks. Austral Vet. Jour. 30:145.

Roberts, R. E.: 1957. Salt tolerance of turkeys. Poultry Sci. 36 672

Rosenberg, M. M., and Tanaka, T.: 1950. Toxicity of chlordane to growing chickens Am. Jour Vet. Res. 11:233.

————, Tanaka, T., and Alder, H. E.: 1950. Toxicity of chlordane to laying pullets Am. Jour Vet. Res. 11:236.

Salisbury, R. M., and Staples, E. L. J.: 1958. Lead poisoning of chickens. New Zealand Vet. Jour. 6.2.

Sanger, V. L., Yacowitz, H., and Moore, E. N.: 1956. Micropathological changes in an experimental hemorrhagic syndrome in chickens fed sulfaquinoxaline and suggested cause of the disease. Am. Jour. Vet. Res. 17:766.

————, Scott, L., Hamdy, A., Gale, C., and Pounden, W D.: 1958 Alimentary toxemia in chickens. Jour. Am. Vet. Med. Assn. 133:172.

Sargeant, K., and O'Kelley, J.: 1961. The assay of a toxic principle in certain groundnut meals. Vet. Rec. 73:1219.

————, Sheridan, A., O'Kelley, J., and Carnaghan, R. B. A.: 1961 Toxicity associated with certain samples of groundnuts. Nature, London 192.1906.

Schmittle, S. C., Edwards, H. M., and Morris, D.: 1958. A disorder of chickens probably due to toxic feed-preliminary report. Jour. Am. Vet. Med. Assn. 132:216.

————, Richey, D. J., and Tumlin, J. L.: 1959. Toxicity of *Crotalaria spectabilis* seed in poultry. Poultry Sci. 38:1244.

Scott, H. M., Jungherr, E. L., and Matterson, L. D.: 1944. The effect of feeding sulfanilamide to the laying fowl. Poultry Sci. 23:446.

Scrivner, L. H.: 1946. Experimental edema and ascites in poults. Jour. Am. Vet. Med. Assn 108:27.

Selye, H.: 1943. Production of nephrosclerosis in fowl by sodium chloride. Jour. Am. Vet. Med Assn. 103:140.

Shaw, P. A.: 1929. Duck disease studies. 11. Feeding of single and mixed salts. Proc. Soc. Exper. Biol. and Med. 27:120.

Shealy, A. L., and Thomas, E. F.: 1928. Daubentonia seed poisoning of poultry. Univ. Fla. Agr. Exper. Sta., Bul. 196.

Sherwin, C. P., and Crowdle, J. H.: 1922. Detoxication in the organism of the fowl. Proc. Soc. Exper. Biol. and Med. 19:518.

Shillinger, J. E., and Cottam, C.: 1937. The importance of lead poisoning in waterfowl. Trans. No. Am. Wildlife Conf. 2:398.

Siller, W. G., and Ostler, D. C.: 1961. The histopathology of an enterohepatic syndrome of turkey poults. Vet. Rec. 73:134.

Simpson, C. F., Pritchard, W. R., and Harms, R. H.: 1959. An endotheliosis in chickens and turkeys caused by an unidentified dietary factor. Jour. Am. Vet. Med. Assn 134:410.

————, Waldroup, P. W., and Harms, R. H.: 1963. Pathologic changes associated with feeding various levels of *Crotalaria spectabilis* seeds to poultry. Jour. Am. Vet. Med. Assn. 142:264.

Smith, F. H., and Osborne, C. J.: 1962. Toxic effects of crotalaria seed. Vet. Med. 57:234.

Stevens, A. J., Saunders, C. N., Spence, J. B., and Newnham, A. G.: 1960. Investigations into "disease" of turkey poults. Vet. Rec. 72:627.

Stiles, D. F.: 1940a. Lead poisoning in ducks of southwestern Iowa during the winter of 1938-39. Proc. Iowa Acad. Sci. 47:597.

————: 1940b. Carbon monoxide poisoning of chicks and poults in poorly ventilated brooders. Poultry Sci. 19:311.

————: 1942. Poisoning of turkey poults from whorled milkweed (*Asclepias galioides*). Poultry Sci 21:263.

Stockman, R.: 1931. The poisonous principle of Lathyrus and some other leguminous seeds Jour. Hyg. 31:550.

————: 1934. The chemistry and pharmacology of Lathyrus peas. Jour. Hyg. 34:145.

Swanson, M. H., Waible, P. E., Heibacka, N. V., and Johnson, E. L.: 1956. Shell egg quality as affected by Arasan in the diet. Poultry Sci. 35-92.

Temperton, H.: 1944. Effect of green and sprouted potatoes on laying pullets. Vet. Med. 39.13.

Thomas, E. F.: 1934. The toxicity of certain species of crotalaria seed for the chicken, quail, turkey, and dove. Jour. Am. Vet. Med. Assn. 85:617.

Thomas, E. W., Neal, W. M., and Ahmann, C. F.. 1935. Toxicity of *Crotalaria spectabilis* Roth to livestock and poultry. Jour. Am. Soc. Agron. 27 499.

Thompson, J. J., and Smith, N. C.: 1953. Exudative diathesis in chicks in New Zealand. Austral. Vet. Jour. 29.89.

Torrey, J. P., and Graham, R.: 1935. A note on experimental salt poisoning in ducks. Cornell Vet. 25:50.

Van Zyl, J. P.: 1929. Annual Report of the Director of Veterinary Services, Union of South Africa 15:1189.

Waibble, P. E., Pomeroy, B. S., and Johnson, E. L. 1955. Effect of Arasan-treated corn on laying hens Science 121:401.

Wannop, C. C. 1960 Disease of turkey poults. Vet Rec. 72:671.

———. 1961 The histopathology of turkey "X" disease in Great Britain. Avian Dis. 5:371.

———, and Chubb, L. G.: 1961. Possible fat intoxication in chickens. Vet. Rec. 73:586.

Weber, A. L., Beaudette, F. R., and Hudson, C. B.: 1932. Arsenic poisoning in poultry. No. Am Vet. 13.46

West, J. L. 1957. Disinfectant poisoning in chicks Vet. Med. 52:40.

Wetmore, A: 1922. Lead poisoning in waterfowl. U.S.D.A., Bul. 793.

Whitehead, F. E.: 1934. The effect of arsenic, as used in poisoning grasshoppers, upon birds. Okla. Agr. Exper Sta Bul. 218

Wickware, A. B. 1910. Lead poisoning in ducks following ingestion of shot. Canad. Jour. Comp Med. 4:201.

———. 1945 Grasshoppers. A potential danger to turkeys. Canad. Jour. Comp. Med. 9.80.

Wilson, H. F. and Holmes, C. E. 1936 Effect on chickens of arsenic in grasshopper bait. Jour. Econ. Entom. 29:1008.

Winchell, C. W: 1925. The use of calcium cyanide in the killing of condemned chickens. Rural New Yorker 84:76.

Witter, J. F.: 1958. A preliminary report on the injurious effect of sodium bicarbonate in chicks. Poultry Sci. 15:256

Wootton, J. C., Artman, N R., and Alexander, J. C: 1962. Isolation of three hydropericardium-producing factors from a toxic fat Jour of the Assn. of Off Agr. Chem, 45:739.

Yacowitz, H., Ross, E, Sanger, V. L., Moore, E. N., and Carter, R. D.: 1955. Hemorrhagic syndrome in chicks fed normal rations supplemented with sulfaquinoxaline. Proc. Soc. Exper. Biol and Med. 89:1.

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41

Diseases of the Turkey**

Dietary Diseases

The turkey responds differently to many of the nutritional factors than does the chicken, and for this reason the requirements are often different. Likewise, the pathological changes seen when these factors are lacking may vary from those seen in the chicken under similar conditions. Only the pertinent facts and differences are given in this section. For more complete information on the nutritional requirements of birds, the reader is referred to the sections on nutrition and vitamin requirements, and to textbooks on turkey

rearing such as Marsden and Martin (1955).

A number of vitamins are known to be needed by turkeys. They are especially important in the starting ration because poults have a higher requirement for some of the vitamins than do baby chicks.

Most vitamins required by turkeys are present in the commonly used feedstuffs, and it is not necessary to purchase them in purified form or in expensive proprietary mixtures. Vitamins A, D, and G (riboflavin) are most likely to be deficient in turkey rations. Table 41.1, compiled by Kratzer (1958), summarizes the symptoms of vitamin deficiencies, the amounts required for normal growth, and the principal sources of the vitamins required by turkeys.

VITAMIN A DEFICIENCY

Sources of vitamin A activity for turkey rations include fish oils, alfalfa meal, yellow corn, fresh greens, and dry vitamin A

TABLE 41.1
Vitamin Requirements of Turkeys (Kratzer, 1958)

Vitamin	Signs of Deficiency	Amount Recommended Per Pound of Feed	Best Sources
A *	Poults—poor growth, unsteady gait, watery eyes, inner eyelid cloudy and partially drawn, white exudate from eyes and sinuses, pustules in mouth and esophagus, white flaky exudate in bursa of Fabricius, high mortality Breeders—loss in weight, unsteady gait, watery eyes, inner eyelid cloudy and partially drawn, white exudate from eyes and sinuses, pustules in mouth and esophagus, poor egg production, poor hatchability with an increase in abnormal embryos	Poults, 4,000 U.S.P. units. Breeders, 4,000 U.S.P. units.	Fresh greens, alfalfa meal, fish oils, commercial concentrates, and synthetic vitamin A.
D₁ *	Poults—rickets characterized by poor growth, leg weakness, enlarged hocks, crooked keel, beaded ribs, soft bones and beak, high mortality. Breeders—low egg production, low hatchability, soft-shelled eggs	Poults, 600 I.C. units. Breeders, 600 I.C. units	Fish oils, activated animal sterols
E *	Development of enlarged hocks in poults fed special rations may be prevented by vitamin E Poor hatchability in breeders Embryos from deficient hens show eye disorders	Poults, 6 I.U. Breeders, 20 I.U.	Fresh greens, grains, alfalfa meal, and commercial concentrates.
K	Blood fails to clot normally Small injuries cause excessive bleeding	Practical rations contain adequate amounts.	Fresh greens, alfalfa meal, and synthetic vitamin K.
Thiamine B₁	Poor growth, emaciation, weakness, inability to stand, and death in poults	Poults, 1 milligram.	Grains, grain by-products, oil cake meals, and synthetic thiamine.
Riboflavin *	Poor growth, leg weakness, low hatchability in breeders	Poults, 2 milligrams. Breeders, 1 8 milligrams.	Milk products, liver meal, fresh greens, alfalfa meal, yeast, fermentation products, synthetic riboflavin.
Pantothenic Acid *	Poor growth, high mortality, and dermatitis at corners of mouth of poults Poor egg production and hatchability in breeders	Poults, 6 milligrams. Breeders, 8 milligrams.	Yeast, molasses, liver meal, milk products, alfalfa meal, wheat bran, fermentation products, synthetic pantothenic acid.
B₆ group	Poor growth, uncoordinated movements, convulsions, high mortality, and poor hatchability in breeders.	Poults, 2 milligrams.	Grain, grain by-products, soybean oil meal, milk products, yeast, alfalfa meal, animal products.
Niacin *	Poor growth, inflammation of the mouth, poor feathering, and perosis	Poults, 35 milligrams Breeders, 15 milligrams.	Wheat and wheat by-products, yeast, liver meal, fish meal, meat scraps, synthetic niacin.
Biotin	Poor growth, dermatitis, perosis, high mortality in poults Poor hatchability.	Exact requirement unknown.	Fresh greens, alfalfa meal, soybean oil meal, cane molasses, grain, grain by-products.
Folic Acid	Poor growth, cervical paralysis, mild anemia, high mortality. Poor hatchability in breeders.	Poults, 0 5 milligrams. Breeders, 0.4 milligrams.	Fresh greens, alfalfa meal, liver meal, wheat bran, soybean oil meal, synthetic folic acid.

(table continued on next page)
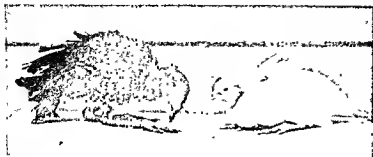
FIG. 41.1 — A 5-week-old poult and a 6-week-old chick, both showing typical signs of vitamin A deficiency. (Hinshaw, Univ. of Calif.)

observed an outbreak of the disease, in a breeding flock, which resulted from feeding dehydrated alfalfa meal low in carotene. In addition to the typical symptoms described by Hinshaw and Lloyd (1934), hatchability of eggs laid by the flock was greatly reduced and poults that did hatch suffered a heavy mortality. The breeders recovered except for blindness, hatchability of eggs increased, and poult mortality decreased after the flock was given high potency vitamin A oil.

Necropsy findings. Lesions are confined principally to the upper digestive tract and to the head. They consist of swollen caseated glands (pustules) in the posterior part of the mouth (Figs. 41.2 and 41.3), the upper esophagus, and the crop, and an involvement of the sinuses of the head (Figs. 41.4 and 41.5). The bursa of Fabricius, an accessory pouchlike organ present only in young poults, located dorsal to the rectum and having an opening into the cloaca, is usually filled with a white, flaky exudate. Urate deposits on the intestines, heart, and lungs, and swollen kidneys filled with urates, common in chickens suffering from vitamin A deficiency, have not been observed in turkeys.

By means of chemical tests available in many diagnostic laboratories, it is possible to determine if turkeys have adequate storage of vitamin A in their bodies. These tests are based on the determination of pro-vitamin A or vitamin A in the livers and may prove an aid in differentiating

FIG. 41.2 — Portion of esophagus of a turkey hen, showing pustular lesions in vitamin A deficiency. (Hinshaw, Univ. of Calif.)
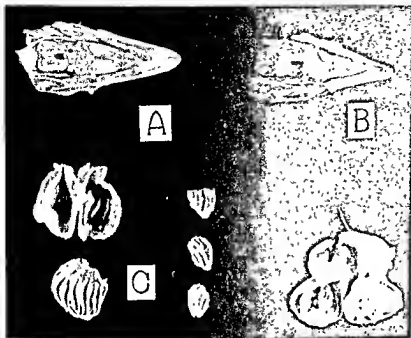
FIG. 41.3.—(A) Floor of mouth and pharyngeal region of a 40-day-old turkey that died from vitamin A deficiency. (B) Some of a 45-day-old chick; note the large number of pustules in B as compared with A. The specimens are typical for the two species. (C) Sagittal section of bursas of Fabricius and caseous plugs characteristic of vitamin A deficiency in young turkeys and chickens; the left bursa was from a poult; the right, from a chick; the middle specimens are typical caseous plugs from bursas taken from chicks. (Hinshaw, Univ. of Calif.)

avitaminosis A from diseases showing similar clinical signs.

Control and prevention. Control and prevention consist of furnishing sufficient vitamin A in the ration. This can be supplied by large amounts of bulky feeds such as fresh greens and alfalfa meal. (See

FIG. 41.4.—Turkey hen showing typical signs of a vitamin A deficiency. Taken 87 days after being on a deficient ration. The hen died 3 days after the picture was taken. (Hinshaw, Univ. of Calif.)

Tables 41.1 and 41.2.) According to the National Research Council, both poults and mature turkeys should receive 4,000 units of vitamin A per pound of feed. It should be noted that the vitamin A of fish oils tends to diminish after the oil has been mixed in the mash, and that when alfalfa meal is stored its vitamin A content decreases, especially in warm weather.

RICKETS (VITAMIN D DEFICIENCY)

Rickets is caused by failure to receive a proper balance of vitamin D_3 and minerals. Vitamin D_3 is also necessary for egg production and hatchability. A lack of it contributes to the development of crooked breast bones. This vitamin is present in certain fish oils. It is also supplied by direct sunlight, which changes certain substances in the skin to vitamin D_3. Vitamin D_3 is of great importance to poults, which have a high requirement.

Signs. Leg weakness, awkwardness of gait, softness of the beak and leg bones, and ruffled, unkempt feathers are characteristic of this condition. The affected poults fail to gain weight and finally die if the balance of minerals and vitamin D_3 is not cor-

FIG. 41.5 – An extreme case of sinusitis in a turkey hen suffering from vitamin A deficiency after being fed for 8 months on a ration containing a low level of vitamin A. The picture on the left (A) is a sagittal section of the head shown on the right. (B) Note the massive accumulation of the whitish-yellow caseous exudate typical of sinusitis as associated with vitamin A deficiency in turkeys. (Hinshaw, Univ. of Calif.)

rected. Vitamin D deficiency in breeders causes lowered egg production, lowered hatchability of eggs laid, and an increase in soft-shelled eggs.

According to Scott et al. (1932), poults receiving a diet deficient only in vitamin D_3 will develop signs in 18 to 20 days, and 100 per cent mortality will occur within 30 days after hatching.

Necropsy findings. Softness of the bony

structures and beading of the ribs are the most common necropsy findings. A definite diagnosis depends on a chemical analysis of the bones or blood or upon the "line" test for rickets.

Prevention and control. There are several forms of vitamin D, some of which are very effective for the prevention of rickets in rats, but not in chicks and poults. For this reason, a source of vitamin

TABLE 41.2
EXAMPLES OF VITAMIN A CONTENT IN FEEDSTUFFS (ASMUNDSON AND KRATZER, 1951)

Feedstuff	Approximate Units of Vitamin A Per Pound	Per Cent in Ration To Supply 4,000 Units Per Pound	Amount in Pounds Per Ton of Ration
Dry "A" concentrate (4,000 A per gram)	1,816,000	0 22	4.4
Fish oil (2,250 A per gram)	1,021,500	0 39	7.8
Alfalfa meal	67,000	6	120 0
Fresh green leaves	45,000	9	180.0
Yellow corn	3,100	50 per cent would supply only 1,500 units	

D_3 such as fish oil should be tested with chicks before use in turkey feeding. Poults need several times as much vitamin D in their feed as chicks (see Table 41.1). The International Chick Unit (ICU) based on vitamin D_3 is now used as the standard unit for poultry feed formulas. The recommended allowance for turkeys is 600 ICU for both poults and breeders. The requirements for poults are several times greater than for chicks, so chick rations should not be fed to poults without adjusting the vitamin D_3 level.

Biologically tested fish oils of guaranteed vitamin D_3 potency are the only oils that are suitable for supplying turkeys with vitamin D_3. Fish oils that have no guarantee of potency may or may not contain adequate vitamin D. Vitamin D_3 activated sterols may be substituted for fish oils (see Table 41.1). Dry products containing synthetic vitamin D_3 or feeding oils are the supplements commonly used in turkey rations. Vitamin D_3 is more stable than vitamin A, but its stability is affected by many of the conditions affecting vitamin A, and care must be taken to avoid adding it to premixes unless it is properly stabilized (Grau *et al.*, 1956).

It is a good practice to add vitamin D_3 to the ration of the breeding flock to insure an adequate storage in the egg for development of the embryo and for starting the poult after it is hatched. Since sunshine cannot be depended upon during the brooding season, fish oil should be a regular part of the ration until the poults are put on the range. Whether or not it should then be continued depends on the amount of sunshine available. A proper balance of minerals, especially calcium and phosphorus, is also essential in preventing rickets.

DIETARY DERMATITIS

Patrick *et al.* (1941, 1943) found that biotin is an antidermatitis factor for turkeys. Niacin and pantothenic acid deficiencies (Kratzer, 1958) may also cause mouth inflammation, which must be differentiated. This deficiency disease may be seen in the field, but other forms of dermatitis are also common and must be considered in making a diagnosis. Very little is known regarding the other types of dermatitis (see Miscellaneous Diseases).

Signs. The clinical signs of dietary dermatitis in poults consist of a sore mouth and encrustations at the corners of the mouth; diarrhea, resulting in an inflamed encrusted vent; thickened eyelids that tend to stick together; ragged feathers; and a listless, unthrifty appearance. In advanced cases the feet may also be involved (Fig. 41.6A and B). Growth is very slow, and mortality is high.

Pantothenic acid (filtrate factor) is sometimes spoken of as the "chick antidermatitis vitamin." Dermatitis of a mild form is seen in poults fed a diet deficient in this ration, according to Kratzer and Williams (1948). Such poults grow more slowly than normal and suffer a heavier mortality than chicks fed the same deficient diet.

Prevention of dietary dermatitis consists in supplying adequate amounts of the vitamins concerned. The important sources of these and amounts needed are given in Table 41.1.

PEROSIS (Slipped Tendon, Hock Disease, Spraddle Legs)

Perosis (Figs. 41.7, 41.8, and 41.9) may cause considerable loss to turkey growers if not prevented by use of a properly balanced ration. Jukes (1940) and Evans *et al.* (1943) have shown that this condition in turkeys is associated with an improper balance of calcium, phosphorus, manganese, and choline in the ration. According to Patrick *et al.* (1943), biotin is also an antiperosis factor, and according to Briggs (1946) and Jukes *et al.* (1947), niacin is necessary to prevent it. Lack of manganese is probably the most common cause.

This disease should not be confused with a similar condition of newly hatched poults which is also called "spraddle legs." This latter condition is caused by one of a number of factors including faulty in-
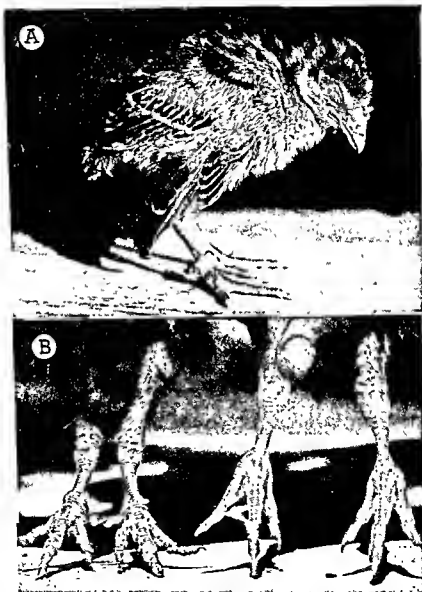
FIG. 41.6 — (A) A 29-day-old turkey after 17 days on a deficient diet. Note the encrusted eyelids, mouth, and nostrils. The feet did not show lesions at the time this picture was taken. (B) Legs and feet of two 3-week-old turkeys. The ones on the left are from a poult fed a deficient ration from hatching time. The others are from a poult fed a normal ration. Note the dryness of the skin of the legs and the marked ulceration of the foot pads in the affected specimen. (T. H. Jukes.)
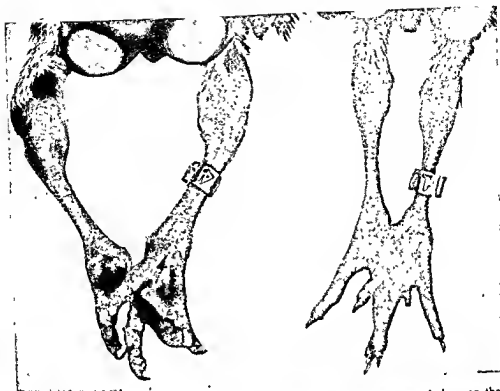
FIG. 41.7 — Perosis. Note thickening, shortening, and distortion of the perotic legs on the left as compared with the normal legs on the right. (T. H. Jukes, Jour. of Nutr.)
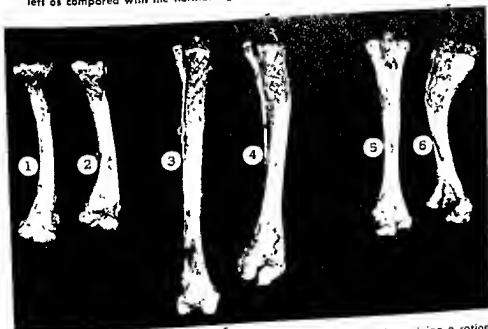
FIG. 41.8 — Leg bones (2, 4, 6) from a perotic 4-week-old turkey poult receiving a ration deficient in choline, compared with leg bones (1, 3, 5) from a normal poult. Note the shortening, thickening, and distortion of the bones caused by choline deficiency. (T. H Jukes, Jour. of Nutr.)

FIG. 41.9 — An advanced case of slipped tendon in a mature turkey. Note the rotation of the right leg at the hock joint. (Hinshaw, Univ. of Calif.)

cubation, improper diet in breeding stock, and faulty structure of hatching trays. Staphylococcosis should also be differentiated from this type of deformity.

Signs. The signs seen in this disease are bowed or badly twisted legs due to improper calcification of the tibia and metatarsus, especially at the hock joints. This deformity allows the tendon of Achilles to slip from its groove. In turkeys the metatarsus often turns at a right angle, giving the name "spraddle legs" to the condition. Occasionally, the femorotibial joint is affected. There is usually enlargement and flattening of the hock joint, and sometimes the entire shank.

Prevention. There is no cure for the disease after it reaches the deformity stages. According to Asmundson and Kratzer

(1951), a poult ration containing from 0.8 to 1.0 per cent phosphorus and 1.8 to 2.0 per cent calcium may be relied upon to provide enough calcium and phosphorus for bone formation and at the same time prevent perosis under ordinary conditions.

Hopper feeding of limestone grit to growing turkeys is not recommended as this practice is unnecessary and dangerous since it upsets the mineral balance of the ration. If grit is to be supplied, it should be of an insoluble type. A summary of the sources of the vitamins needed to prevent perosis and the amounts needed are given in Table 41.1.

MISCELLANEOUS DEFICIENCIES

A number of other deficiencies may occur in turkeys, and the reader is referred to the section on nutrition and vitamin requirements for details. References to a few are given below.

Lysine deficiency in poults is characterized by a white bar appearing on the feathers of bronze and other dark-feathered breeds. Descriptions of this deficiency disease have been reported by Fritz et al. (1947), German et al. (1949), Kratzer et al. (1950), and Grau et al. (1958).

This is the only amino acid studied to date which produces any easily recognized symptom except poor growth. Figure 6.1 shows the effects of a deficiency of this amino acid on the feathers.

Scott (1950), 1951a and b) described a deformity in poults characterized by a swelling of the tibiometatarsal joint associated with a failure in retention of creatinine. This swelling frequently appeared in 2-week-old poults and recurred when the poults were 14 to 16 weeks of age. Retention of creatinine was increased by enriching the ration with vitamin E and inositol. This condition should not be confused with staphylococcosis. The deficiency could, however, predispose poults to staphylococcosis. An enlarged hock condition associated with an isolated soybean protein has been described by Hunt and McGinnis (1959). This condition was prevented by washing the protein concentrate

with water (60° C.) or by addition of all vitamins at a 15-fold increase over the basic level.

Riboflavin deficiency causes poor growth, leg weakness, and poor hatchability of eggs. Table 41.1 and Chapter 3 should be consulted for additional information. Figure 7.12 illustrates the deficiency.

A deficiency of folic acid causes poults to develop a type of cervical paralysis which usually results in death, according to Richardson *et al.* (1945) and Jukes *et al.* (1947). Signs of this deficiency are described in Chapter 7. Figure 7.17 illustrates the cervical paralysis seen.

For the effects of various mineral deficiencies the reader is referred to Chapter 6. A number of papers have been published recently on the effects of zinc deficiency in turkeys. Good reviews on this subject will be found in reports by Supplee *et al.* (1961) and Sullivan (1961a and b).

REFERENCES

Asmundson, V. S., and Kratzer, F. H.: 1951. Turkey production in California. Calif. Agr. Exper. Sta, Ext. Circ. 110.

———, and Kratzer, F. H.: 1952. Observations on vitamin A deficiency in turkey breeding stock. Poultry Sci. 31:71.

Bieter, B. W.: 1956. Keratoconjunctivitis in turkeys — a preliminary report. Vet. Med. 51:363.

Briggs, G. M.: 1946. Nicotinic acid deficiency in poults and the occurrence of perosis. Jour. Nutr. 31:79.

Evans, R. J., Rhian, M., and Draper, C. I.: 1943. Perosis in turkey poults and the choline content of their diets. Poultry Sci. 22:88.

Fritz, J. C., Halpin, J. L., and Hooper, J. H.: 1947. Studies on the nutritional requirements of poults. Poultry Sci. 26:78.

German, H. L., Schweigert, B. S., Sherwood, R. M., and James, L. E.: 1949. Further evidence of the role of lysine in the formation of normal bronze turkey feathers. Poultry Sci. 28:165.

Grau, C. R., Kratzer, F. H., and Newlon, W. E.: 1956. Principles of nutrition for chickens and turkeys. Calif. Agr. Exper. Sta., Ext. Circ. 450.

Hinshaw, W. R., and Lloyd, W. E.: 1934. Vitamin A deficiency in turkeys. Hilgardia (Univ. of Calif.) 8:281.

Hunt, J. R., and McGinnis, J.: 1959. The prevention of a perosis-like condition in turkey poults. Poultry Sci. 38 612.

Jukes, T. H.: 1940. Effect of choline and other supplements on perosis. Jour. Nutr. 20:445.

———, Stokstad, E. L. R., and Belt, M.: 1947. Deficiencies of certain vitamins as studied with turkey poults on a purified diet. I. Pteroylglutamic acid, riboflavin, niacin, and inositol. Jour. Nutr. 33:1.

Kratzer, F. H.: 1958. Vitamin requirements of turkeys. Turkey World 33:69 (Jan).

———, and Williams, D.: 1948. The pantothenic acid requirement of poults for early growth. Poultry Sci. 27 518.

———, Williams, D., and Marshall, B.: 1950. The relation of lysine and protein level in the ration to the development of feather pigment in turkey poults. Poultry Sci. 29:285.

Marsden, S. J., and Martin, J. H.: 1955. Turkey Management, 6th Ed. The Interstate, Danville, Ill. P. 999.

Moore, E. E.: 1958. *Aspergillus fumigatus* as a cause of ophthalmitis in turkeys. Poultry Sci. 32:796.

Patrick, H., Boucher, R. V., Dutcher, R. A., and Knandel, H. C.: 1941. Biotin and prevention of dermatitis in turkey poults. Proc. Soc. Exper. Biol. and Med. 48:456.

———, Boucher, R. V., Dutcher, R. A., and Knandel, H. C.: 1943. Prevention of perosis and dermatitis in turkey poults. Jour. Nutr. 26:197.

Richardson, L. R., Hogan, A. G., and Kempster, H. L.: 1945. Requirement of turkey poults for vitamin B₁. Jour. Nutr. 30:151.

Scott, H. M.: 1937. Turkey production in Kansas. Kans Agr. Exper. Sta., Bul. 276.

———, Hughes, J. S., and Loy, H. W.: 1932. Rickets in young turkeys. Poultry Sci. 11:177.

Scott, M. L.: 1950. Studies on the enlarged hock disorder (perosis) in turkeys. Jour. Nutr. 40 611.

———: 1951a. Studies on the enlarged hock disorder in turkeys. 2. Factors affecting the excretion and retention of creatine by young poults. Poultry Sci. 30 839.

———: 1951b Studies on the enlarged hock disorder in turkeys. 3. Evidence of the detrimental effect of fish liver oil and the beneficial effect of dried brewers' yeast and other materials. Poultry Sci. 30.846.

Stoewsand, G. S., and Scott, M. L.: 1961. The vitamin A requirements of breeding turkeys and their progeny. Poultry Sci. 40:1255.

Sullivan, T. W.: 1961a. The zinc requirement of broad-breasted bronze poults. Poultry Sci. 40:354.

Sullivan, T. W.: 1961b. The availability of zinc in various compounds to broad-breasted bronze poults. Poultry Sci. 40:340.

———. 1961b. The availability of zinc in various compounds to broad-breasted bronze poults. Poultry Sci. 40:340.

Supplee, W. C., Creek, R. D., Combs, G. F., and Blamberg, D. L.: 1961. The zinc requirements of poults receiving practical diets. Poultry Sci. 40:171.

Wilgus, H. S.: 1940. Experiments show turkey poults need four times as much vitamin A as do chicks. Colo. Agr. Exper. Sta., Bul. 2:3.

Fungus Diseases

Fungus diseases, due to molds and yeasts, may cause considerable mortality in turkeys. The most important are aspergillosis, favus, and candidiasis. For an excellent bibliography on avian mycosis see Chute et al. (1962). For a comprehensive review on mycotoxicosis see Forgacs and Carll (1962). See also Chapter 18.

ASPERGILLOSIS (Brooder Pneumonia, Mycotic Pneumonia, Pneumomycosis)

Aspergillosis, more commonly known as brooder pneumonia, is caused principally by *Aspergillus fumigatus*, although other molds of the same genus may be responsible. *A. fumigatus* is widely distributed in nature and is pathogenic for many animals, including man. In young poults kept on contaminated litter, it produces pneumonia with heavy mortality. Infected older birds may suffer from pneumonia or air-sac infection.

Aspergillosis has been reported in turkeys by Lignières and Petit (1898), Balfour (1911), Schlegel (1915), Durant and Tucker (1935), Hinshaw (1937), Witter and Chute (1952), Moore (1953), and Raines et al. (1956). The outbreak reported by Durant and Tucker occurred in wild turkey poults reared in captivity. The disease appeared at 5 days of age and reached a maximum mortality at 15 days. When the epizootic subsided at the end of 3 weeks, only 200 of the 785 poults remained alive. Moore reported ophthalmitis as a common symptom in outbreaks observed by him, and Raines et al. described encephalitis as a common manifestation in an outbreak of 18-day-old poults. Van Heelsbergen (1929) gives a detailed description of an outbreak investigated by Schlegel. The description given below, taken from Hinshaw (1937), is essentially the same as described by Schlegel and is based on the writer's experience with the disease.

Signs. The signs depend on the location of infection. Lesions in the mouth, trachea, or bronchi produce hoarseness, heavy breathing, and sometimes rattling in the throat. As the disease progresses, dullness, labored breathing, and emaciation may be seen. Death probably results from either toxemia or asphyxiation. The mortality varies but is usually greater in brooder poults than in older birds.

Necropsy findings. Diagnosis is readily made in advanced cases. The lungs and air sacs are the principal seats of infection, but the process may extend into the peritoneal cavity or into the air passages of the bones (Fig. 41.10). The kidneys, liver, and spleen may be affected by direct contact from the air sacs. Yellow, semiliquid, or caseated masses in the air sacs and lungs, with buttonlike ulcers attached to the mucous membranes, are common. In the early stages these ulcers appear as round, yellowish-white masses attached to the membrane. In advanced cases a greenish mold turf may be seen over the surfaces of the infected areas and in the convex depressions of the ulcers, especially in the air sacs.

In cases of ophthalmitis, described by Moore (1953), the primary involvement was in the vitreous humor and the adjoining tissues. In one bird he observed the presence of mycelia in the crystalline lens.

In encephalitis aspergillosis described by Raines et al. (1956), torticollis and lack of equilibrium were observed. The brains of such cases contained necrotic foci 1–2 mm. in diameter in both the cerebellum and cerebrum. Hubben (1958) has reported meningoencephalitis in both turkeys and ducks. A mycotic encephalomalacia has

FIG. 41.10 — Lungs of turkey showing typical caseous nodules seen in the early stages of aspergillosis. Note also irregular lesion with center darkened by aerial hyphae of the fungus, denoted by the arrow. (Hinshaw, Univ. of Calif.)

been described by Jungherr and Gifford (1944).

Final diagnosis depends on identification of the mold. The fungus can be readily demonstrated by microscopic examination of specimens that have been treated with 10 per cent sodium or potassium hydroxide and by culturing on suitable media. A careful examination of the surface of the buttonlike lesions will often reveal aerial hyphae, and seedings from these will usually insure a pure culture. See also the section on Mycotoxicosis.

Prevention, control, and treatment. Careful selection of mash, grain, and litter is essential in preventing this disease. Access to musty, moldy strawstacks should be avoided.

Improperly kept drinking fountains used for dispensing milk have been found to be a source of infection. One outbreak was associated with contaminated milk cans. The inside of the lids of the cans used for transporting milk was found to be covered with a fine mold growth; the owner had washed and scalded the cans daily but thought it unnecessary to clean the lids. The storage barrels for the milk were also heavily infected.

The areas around feed hoppers and watering places are fertile fields for the growth of molds. Unless a permanent yard

system is used, frequent moving of feed troughs and watering places is advisable. Placing feed containers and watering fountains on screened elevated platforms helps to prevent turkeys from picking up molds that develop in such places. Drainage is advisable for areas where water is liable to stand after rains.

Control is best accomplished by removing the cause. A careful search should be made for mold in the litter, the feed, and the feed and water containers. Daily cleaning and disinfection of feed and water utensils will aid in eliminating the infection. Spraying of the ground around the containers with chemical solutions may be advisable if it is impossible to change feeding areas frequently. In outbreaks, a 1:2,000 solution of copper sulfate in place of all drinking water may be used to aid in preventing the spread through this means, though it should not be relied upon as a preventive to be used continually. The antifungal antibiotics as preventives for this disease should be considered.

The deep-seated nature of the respiratory form of the disease renders treatment of little avail. *Extreme care should be used in handling and disposing of sick birds because of the possible danger of transmitting the disease to the attendant.*

MYCOTOXICOSIS

Mycotoxicosis is a poisoning of the host which follows the entrance into the body of toxic substances of fungal origin. A large number of fungi are capable of producing toxins and the reader is referred to Forgacs and Carll (1962) for a comprehensive review.

Peanut (ground nut) meal poisoning (Turkey X Disease). After the review by Forgacs and Carll (1962) was written and in the process of publication, a serious disease of turkeys which was causing enormous losses on British turkey farms was reported. This disease affecting all ages was called Turkey X Disease by Blount (1961) and was subsequently found to be caused by a toxin produced by *Aspergillus flavus* (Sargeant *et al*, 1961). The source was contaminated peanut (ground nut) meal used widely in British animal feeds. Over 500 British turkey farms were affected in 1960 and losses in excess of 100,000 turkeys were reported.

Other pertinent references on these outbreaks and subsequent reports on research on the disease in turkeys include the following. Asplin and Carnaghan (1961), Lancaster *et al* (1961), Siller and Ostler (1961), Wannop (1961), Allcroft and Carnaghan (1962), and Nesbit *et al* (1962). Chickens and ducks were also found to be susceptible, and ducklings were found to be the laboratory animal of choice for detecting toxicity of suspected toxin-containing feeds. Blount *et al* (1963) found differences in susceptibility of different strains of ducklings used for assay. It should be emphasized that other animals are also susceptible to this toxin (Clegg, 1962) and that *A. flavus* can contaminate other substances used in animal feeds (Richmond *et al*, 1962). Toxins from other species must also be considered (Forgacs and Carll, 1962).

Signs. The signs of the disease as described by the various investigators include death without observed signs, depression, unsteady gait, stiffness of the muscles, and torticollis. Falling over backwards was also observed.

Necropsy findings. On necropsy, typical lesions of the kidneys include membranous glomerulonephritis and hyaline droplet nephrosis. The liver shows a very severe hepatic necrosis and excessive bile production. Marked catarrhal enteritis especially in the duodenum is characteristic.

Treatment. No specific treatment has been suggested. Forgacs *et al*. (1963) have, however, reported on the antimycotoxic activity of 8-hydroxyquinoline under laboratory and simulated field conditions with a number of mycotoxins. The British outbreaks have stimulated a large number of investigations aimed at producing fungus-free sources of peanuts used for production of meal for use in animal feed rations.

Hemorrhagic syndrome. Although this well known condition in chickens and especially in broilers (Forgacs *et al.*, 1958, 1962) has not been reported in turkeys, the possibility of finding it in turkeys should not be overlooked.

FAVUS

Favus is a chronic skin disease caused by a fungus, *Achorion gallinae*, and characterized by whitish areas on the exposed skin parts of the body (Fig. 41.11). It is not a commonly occurring disease. *Since man is susceptible, care should be taken to prevent transmission if an outbreak occurs*. The disease is generally mild and sporadic in nature. It may exist in a flock for several months, but few losses directly traceable to it are experienced.

Signs. The white powderlike spots which characterize the disease usually appear first around the beak. Thence they spread to the wattles, dewlap, and snood, and in extreme cases to the feathered portions. The fine pinpoint white spots finally coalesce and may cover a considerable area. As the fungus spreads and grows, a piling up of the threads occurs, and a thick, crustlike area may result.

Prevention, control, and treatment. Removal and disposal of all infected birds is recommended. It is well to move the flock to new quarters when practicable. After removal of infected individuals, the

FIG. 41.11 — Favus. An unusual case affecting the entire carcass. (L. D. Bushnell.)

premises must be thoroughly cleaned and disinfected.

Treatment should be attempted only in very valuable birds. Fungicidal ointments and antifungal antibiotics may be of value, but no experimental information on their efficacy is available. A mixture of 6 parts of glycerine and 1 part of iodine applied locally is recommended by van Heelsbergen (1929) for the infected parts of the head. Beach and Halpin (1918) reported that a formalin-vaseline ointment rubbed thoroughly into the lesions cured 50 out of 52 cases treated.

CANDIDIASIS (Mycosis of the Crop, Moniliasis, Thrush)

Candidiasis is a disease of the upper digestive tract of both chickens and turkeys caused by yeastlike organisms belonging to the genus, Candida. Jungherr (1933a) was probably the first in the United States to observe the disease in chicks; Gierke (1932) has reported an outbreak of a thrushlike disease occurring in turkeys in California during the summer of 1932; and Hinshaw (1933) has described the results of studies on several outbreaks in turkeys and chickens. A review on the disease as it now exists in turkeys and chickens in California is recorded by

Mayeda (1961). Hart (1947) reported the disease in turkeys and other fowl in New South Wales. Blaxland and Fincham (1950) and Jordan (1953) have reported the existence of the disease in Great Britain. Wickerham and Rettger (1939), in a taxonomic study of Candida species from various sources, included several strains isolated by Hinshaw from turkeys and chickens. These proved to be Candida albicans and were indistinguishable from species isolated from man. Jungherr (1933b, 1931) found in later studies that C. albicans and C. krusei and Oidium pullorum n.sp. were the yeastlike fungi most frequently isolated from chicks. Of these he considered C. albicans and O. pullorum of etiological importance. A soluble endotoxin, toxic for mice, was isolated from C. albicans by Salvin (1952). As far as is known, the influence of this endotoxin on the disease in fowl has not been investigated.

Jungherr (1933a) was able to transmit the disease in chicks by feeding fecal material from diseased chicks and by injecting pure cultures of C. albicans. The average period of incubation under experimental conditions was 31 days. Hinshaw (1933) was able to transmit the disease from turkeys to turkeys, chickens, and rabbits. He observed that the disease is one

associated with poor management, where unsanitary surroundings prevail and debilitation is prevalent. Under such conditions the use of antibacterial antibiotics in feed or drinking water could result in an increase in *Candida albicans* in the digestive tract. This in turn could influence the incidence and severity of the disease. The relation of *Candida* sp. to pendulous crop is discussed in the section on that disease.

Signs. As most of the outbreaks observed have been complicated with some other pathologic condition, specific signs have been difficult to determine. More or less constant clinical signs, however, are listlessness, loss of appetite, tendency to stand around with heads drawn back on *the shoulders*, and a sunken appearance of the chest. The eyes and sinuses appear sunken and the heads haggard. Candidiasis must be differentiated from the disease formerly described by Jungherr (1927) as a mycosis of the crop of turkeys but now known to be caused by a Trichomonas. (See Trichomoniasis of Upper Digestive Tract.)

Underwood (1955) has described the use of a panendoscope as aid in diagnosing the disease. This instrument is inserted into the crop via the mouth and esophagus and permits visual examination of the mucous membrane for lesions.

Necropsy findings. The crop has been the most common seat of infection. Fungi have also been demonstrated in scrapings from the mouth, infraorbital sinuses, upper and lower esophagus, proventriculus, gizzard, and intestines. Cultures of the causative organism have been obtained from all of these organs and in addition from a lung abscess and from a skin abscess.

In the more acute cases, as well as in the milder cases, there is seen a catarrhal to thick mucoid exudate with a tendency to form a pseudomembrane. Soft, raised, whitish-yellow ulcers having a roselike appearance and scattered over the surface, at times coalescing to form a solid mass of piled-up exudate (Fig. 41.12) characterize the more chronic cases. These lesions have been variously described by turkey growers and others as having a "turkish-

FIG. 41.12 — (A) Crop of a turkey suffering from candidiasis (moniliasis). (B) Enlarged section of A; note the raised, piled-up exudate which tends to form roselike masses. (Hinshaw, Univ. of Calif.)

towel-like" or "curdy" appearance. In early or mild cases the mucous membrane may appear parboiled. The lesions are easily scraped from the surface, leaving the mucous membrane abraded and injured.

In most cases the crops either are empty or contain a small amount of thick slimy exudate. Cultures of the fungus are readily obtained in nearly pure state by washing off the surface exudate and planting a fairly deep scraping on suitable media.

Prevention, control, and treatment. Because of the nature of the disease and its frequent association with other diseases common in crowded quarters and in flocks suffering from some form of malnutrition, sanitation and proper diet are important factors in control. Removal of birds to clean and thoroughly disinfected quarters, together with the daily cleaning and disinfection of feed and water containers, has helped to reduce losses.

According to Underwood et al. (1956), copper sulfate either in the feed or in drinking water (1:1,000 and 1:1,500) has little effect on C. albicans or the disease in experimentally produced cases in chicks and poults. Yacowitz et al. (1959) reported successful prevention of Candidiasis in chickens by the addition of an antifungal antibiotic, Nystatin (Mycostatin-Merck), at a minimum level of 152 mg. per kilogram of ration for a period of 4 weeks. Kahn and Weisblatt (1963) obtained similar results. Wind and Yacowitz (1960) successfully treated crop mycosis with mystatin (Mycostatin-Squibb) by dispersing it in drinking water at levels of 62.5 to 250 mg. per liter with sodium lauryl sulfate (7.8 to 25 mg. per liter) for a 5-day period.

If antibacterial antibiotics are being used in an infected flock for nutritional purposes, their removal may aid in reducing the Candida flora.

REFERENCES

Allcroft, R., and Carnaghan, R. B. A: 1962. Groundnut toxicity—Aspergillus flavus toxin (aflatoxin) in animal products—preliminary communication. Vet. Record 71:865.
Asplin, F. D., and Carnaghan, R. B. A.: 1961. The toxicary of certain groundnut meals for poultry with special references to their effects on ducklings and chickens. Vet. Record 73:1215.
Balfour, A.: 1911. Aspergillary pneumokoniosis in the lung of a turkey. Fourth Rep. Wellcome Res. Lab. (Gordon Mem. Coll.), Vol. A. (Med.) P. 353.
Beach, B. A., and Halpin, J.: 1918. Observations on an outbreak of favus. Jour. Agr. Res 15:415.
Blaxland, J. D., and Fincham, I. H.: 1950. Mycosis of the crop (moniliasis) in poultry with particular reference to serious mortality occurring in young turkeys. Brit. Vet. Jour. 106:221.
Blount, W. P.: 1961. Turkey "X" Disease. Turkeys (Brit.) 9:52.
———. France, D., McK., Knight, D., and Dowling, W. M.: 1963. The use of ducklings for the detection of aflatoxin. Vet. Record 75:35.
Chute, H. L., O'Meara, D. C., and Barden, E. S.: 1962. A bibliography of avian mycosis (partially annotated). Maine Agr. Exper. Sta. Misc. Pub. 655:1.
Clegg, F. G.: 1962. An outbreak of poisoning in store cattle attributed to Brazilian groundnut meal. Vet. Record 74:992.
Durant, A. J., and Tucker, C. M.: 1935. Aspergillosis of wild turkeys reared in captivity. Jour. Am. Vet. Med. Assn. 86:781.
Forgacs, J., and Carll, W. T.: 1962. Mycotoxicoses. Adv. in Vet. Res. 7:273. (Section of moldy feed toxicosis in poultry on page 316)
———, Koch, H., Carll, W. T., and White-Stevens, R. H.: 1958. Additional studies on the relationship of mycotoxicoses to the poultry hemorrhagic syndrome. Am. Jour. Vet. Res. 19:744.
———, Koch, H., Carll, W. T., and White-Stevens, R. H.: 1962. Mycotoxicoses. I. Relationship of toxic fungi to moldy-feed toxicosis in poultry. Avian Dis. 6:363.
———, Koch, H., and White-Stevens, R. H.: 1963. Mycotoxicoses. III. Antifungal and antimycotoxic activity of 8-hydroxyquinoline under laboratory and simulated field conditions. Avian Dis. 7:56.
Gierke, A. G.: 1932. A preliminary report on a mycosis of turkeys. Calif. St. Dept. Agr. Monthly Bul. 21:229.
Hart, H. L.: 1947. Moniliasis in turkeys and fowls in New South Wales. Austral. Vet. Jour. 23:191.

Hinshaw, W. R.: 1933. Moniliasis (thrush) in turkeys and chickens. Proc. Fifth World's Poultry Cong., Paper 97:1.

———: 1957. Diseases of turkeys. Calif. Agr. Exper. Sta., Bul. 613.

Hubben, K.: 1958. Case report — Aspergillus meningoencephalitis in turkeys and ducks. Avian Dis. 2:110.

Jordan, F. T. W.: 1953. The incidence of Candida albicans in the crops of fowls. Brit. Vet. Jour. 109:527.

Jungherr, E.: 1927. Two interesting turkey diseases. Jour. Am. Vet. Med. Assn. 71:636.

———: 1933a. Observations on a severe outbreak of mycosis in chicks. Jour. Agr. Res. 46:169.

———: 1933b. Studies on yeast-like fungi from gallinaceous birds. Storrs Agr. Exper. Sta., Bul. 183.

———: 1934. Mycosis in fowl caused by yeast-like fungi. Jour. Am. Vet. Med. Assn. 84.500.

——— and Gifford, R.: 1944. Three hitherto unreported turkey diseases in Connecticut, erysipelas, hexamitiasis, mycotic encephalomalacia. Cornell Vet. 34:214.

Kahn, S. G. and Weisblatt, H.: 1963. A comparison of nystatin and copper sulfate in experimental moniliasis of chickens and turkeys. Avian Dis. 7:301.

Lancaster, M. C., Jenkins, F. P., and Philip, J. McL.: 1961. Toxicity associated with certain samples of ground nuts. Nature 192:1096.

Lignières, and Petit: 1898. Péritonite aspergillaire des dindons. Rec. Méd. Vét. 75:145.

Mayeda, B.: 1961. Candidiasis in turkeys and chickens in the Sacramento Valley of California. Avian Dis. 5:232.

Moore, E. N.: 1953. Aspergillus fumigatus as a cause of ophthalmitis in turkeys. Poultry Sci. 32.796.

Nesbitt, D. B., O'Kelly, J., Sargeant, K., and Sheridan, A.: 1962. Toxic metabolites of Aspergillus flavus. Nature 195:1062.

Raines, T. V., Kuzdas, C. D., Winkle, F. H., and Johnson, B. S.: 1956. Encephalitic aspergillosis in turkeys—case report. Jour. Am. Vet. Med. Assn. 129:435.

Richmond, J. W., Sutcliff, N. W., Daniels, N. W. R., Egitt, P. W. R., and Coppock, J. B. M.: 1962. Factors other than groundnut relating to turkey X disease. Vet. Record 74:544.

Salvin, S. B.: 1952. Endotoxin in pathogenic fungi. Jour. Immunol. 69.89.

Sargeant, K., Sheridan, A., O'Kelly, J., and Carnaghan, R. B. A.: 1961. Toxicity associated with certain samples of groundnuts. Nature 192:1096.

Schlegel: 1915. (Quoted by van Heelsbergen.)

Siller, W. G. and Ostler, D. C.: 1961. The histopathology of an enterohepatic syndrome of turkey poults. Vet. Record 73:134.

Underwood, P. C.: 1955. Detection of crop mycosis (moniliasis) in chickens and turkey poults with a panendoscope. Jour. Am. Vet. Med. Assn. 127:229.

———, Collins, J. H., Durbin, C. G., Hodges, F. A., and Zimmerman, H. E.: 1956. Critical tests with copper sulfate for experimental moniliasis (crop mycosis) of chickens and turkeys. Poultry Sci. 35:599.

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart, p. 312.

Wannop, C. G.: 1961. The histopathology of turkey "X" disease in Great Britain. Avian Dis. 5 371.

Wickerham, L. J. and Rettger, L. F.: 1939. A taxonomic study of Monilia albicans with special emphasis on morphology and morphological variation. Jour. Trop. Med. and Hyg. 42:174, 187, and 204.

Wind, S. and Yacowitz, H.: 1960. Use of Mycostatin® in the drinking water for the treatment of crop mycosis in turkeys. Poultry Sci. 59 904.

Witter, J. F., and Chute, H. L.: 1952. Aspergillosis in turkeys. Jour. Am. Vet. Med. Assn. 121:587

Yacowitz, H., Wind, S., Jambor, W. P., Willet, N. P., and Pagano, J. F.: 1959. Use of Mycostatin® for the prevention of moniliasis (crop mycosis) in chicks and turkeys. Poultry Sci. 38.653.

Bacterial and Viral Diseases

The common diseases of turkeys caused by bacterial and viral agents are included in this section. The reader is referred to standard textbooks on bacteriology if descriptions of the causative organisms are desired. For discussions on fowl plague, fowl coryza, ornithosis, equine encephalomyelitis virus in birds, Newcastle disease, and other diseases seldom seen in turkeys,

the reader is referred to the sections on diseases of chickens.

BOTULISM*

Botulism is caused by toxin produced by an anaerobe, Clostridium botulinum. Of the types of botulinic toxins poisonous to man and animals, only A and C are

* See also Chapter 15.

known to affect fowls. The toxins are produced by the microorganism while growing in such substances as decomposing food, dead carcasses, and wet grain, and are transmitted to birds when the contaminated products are eaten. Coburn and Quortrup (1938) described an outbreak of botulism in turkeys caused by the type C organism. The outbreak occurred in a flock of 1,400 turkeys which were ranging on a 20-acre stubble field. About 50 turkeys were sick at the time of the investigation, and an additional 50 had died the previous week. The source of the toxin was found to be a shallow, stagnant pool of water in the stubble field. Filtered water samples from it were shown to contain the type C toxin by tests on white mice. *Clostridium botulinum* (type C) was also isolated from the soil taken from the water hole.

Signs. The most common clinical sign is complete paralysis of the neck, which gives the disease its name, "limberneck." The birds sit with their heads and necks on the ground or extended over the back (Fig. 41.13), often in a comatose condition. In turkeys the feathers do not shed so readily as in chickens affected with the disease.

The turkeys in the outbreaks described by Coburn and Quortrup (1938) manifested evidence of cyanosis of the head, posterior paralysis, and dyspnea, but only a few showed paralysis of the nictitating

membrane, a symptom usually considered pathognomonic. Some of the sick turkeys recovered spontaneously.

Necropsy findings. Coburn and Quortrup described the following postmortem findings: petechial hemorrhages on the auricular pericardium, hyperemia of the duodenal mucosa, and cloaca distended with urates. Thus the gross pathology would make one suspicious of fowl cholera.

One should look for evidence of spoiled food in the crop and for the presence of fly maggots, which are suggestive of the consumption of spoiled food. Diagnosis depends on the history obtained and on the symptoms and necropsy findings, but finally on the demonstration of the toxin or causative organism.

Prevention, control, and treatment. Every effort should be made to prevent turkeys from obtaining foods that might harbor the botulinus organism. Spoiled canned vegetables should never be given, for they are liable to contain botulinus toxin.

When the disease appears, all the birds should be moved to a new feeding ground and, if necessary, fenced to prevent access to spoiled food. Sick birds should have plenty of shade. Their crops can be drained and flushed out with warm water with the aid of a rubber tube and a funnel. Large doses of mineral oil or castor oil may help to get rid of the toxin in birds that have not gone into coma. The cause of the trouble should be traced and recurrence prevented. In valuable birds polyvalent (mixed) botulinus antitoxin may be used.

Persons handling turkeys suffering from botulism should keep in mind that the botulinus toxin may affect man. Careful washing of the hands after care of the birds is suggested as a precautionary measure.

ERYSIPELAS*

This disease, caused by the swine erysipelas organism *Erysipelothrix insidiosa (rhusiopathiae)*, was first reported in tur-

FIG. 41.13 — Typical posture in botulism of turkeys. (Hinshaw, Univ. of Calif.)

* See also Chapter 15.

keys by Jarosch (1905). The first report of an outbreak in the United States was made by Beaudette and Hudson (1936) in New Jersey. Since 1936 it has been diagnosed in various sections of the United States (Hudson, 1949). Reference is made to Chapter 15 as to how the disease affects other birds.

Most of the outbreaks reported in the literature have been confined to one farm with no recurrence the following year. In some areas, however, particularly in northwestern United States, the disease is now enzootic, and recurrence on the same farm is common. Swine and sheep, reported to be important factors in transmission in some areas, do not appear to be the sources in the Northwest. Fish meal has been suggested as a possible source of this infection (Grenci, 1943). The outbreaks have usually occurred in turkeys approaching the market age, and males have appeared to suffer the heaviest losses. Rosenwald and Dickinson (1941) have, however, diagnosed the disease in poults from a few weeks of age to maturity.

Signs and mortality. The disease manifestations are primarily those of a septicemia. The mortality in an outbreak studied by Beaudette and Hudson (1936)

was high, 200 of a flock of 500 dying in 9 days. Often, the first indication of the presence of the disease is the finding of dead birds which appear to be in good condition.

The clinical signs are listlessness, drooping tails and wings, and sometimes yellowish green diarrhea. Swelling of the joints of the legs has been noted, but this is not a constant sign. Affected birds tend to remain aloof from the remainder of the flock. These sick birds crouch; the heads often appear cyanotic, and nasal catarrh is a common sign. Swelling of the snood, as illustrated in Figure 41.14, is characteristic of this disease, but similar swellings are also seen in outbreaks of fowl cholera. Erysipeloid lesions commonly appear on the face, involving the major portion of the eyelids and area posterior to them (Fig. 41.15). The other areas of the skin most often affected are the wattles and breast. In acute outbreaks many of the individuals develop varying degrees of skin necrosis. Vegetative endocarditis may cause listlessness and loss of body flesh of a number of birds in a flock several weeks after the acute stage. The effect on the appetite seems to vary in different outbreaks, though most investigators agree that feed

FIG. 41.14 — Erysipelas. Swollen snood which is, according to Rosenwald and Dickinson, pathognomonic for the disease may also be seen in outbreaks of fowl cholera. (Rosenwald and Dickinson, Am. Jour. Vet. Res.)

consumption is lowered. Recovery in acute cases may be complete in a week or 10 days.

Temperatures as high as 109.6° F. have been noted in field outbreaks of the disease in turkeys, but rise in temperature has not been consistent with clinical signs. Adult turkey hens artificially infected with E. insidiosa have not shown uniformly marked increase in temperature. One such bird (19 weeks of age), given 0.8 cc. of 24-hour broth culture, became ill in 48 hours and did show a continued rise in temperature until it reached 110.6° F. on the fourth day after inoculation. On the day of its death (the sixth after inoculation) the temperature was 109.8° F. Another bird, which became visibly ill in 48 hours but recovered within 2 weeks, showed only a slight rise in temperature during the period of visible symptoms. A third turkey hen showed an increase from 104.9° F. on the day before inoculation to 108.8° F. on the sixth day after inoculation. Its temperature gradually subsided though the bird itself developed a chronic type of the disease and was finally killed in an emaciated condition after 4 weeks.

Necropsy findings. Diffuse hemorrhagic areas of various sizes are common in the breast muscles. The skin of the breast may develop purple-colored, irregular-shaped blotches, commonly referred to as "erysipelas blush." Diamond skin lesions so common in the chronic disease of swine are seldom seen in turkeys, but one such case is described by Peterson and Hymas

(1950). The nasal passages are usually filled with thick mucus; the livers are enlarged, congested, and friable. Catarrhal enteritis is evident, with some reddening of the mucosa of the large intestine. In most cases the spleens are enlarged, mottled, and friable, hemorrhages sometimes appearing. Other lesions occasionally found are hemorrhages in the pericardium, congestion of the kidneys and lungs, and, rarely, browning of the lung tissue. Vegetative endocarditis involving the bicuspid and tricuspid valves is often seen in chronic cases (Fig. 41.16). Stiles (1946) considered the absence of pus in affected joints and other structures as characteristic of the disease.

Diagnosis. The marked hemorrhagic condition of the skin and fascial and muscular tissues of the breast is the most significant finding at necropsy according to most investigators. Diagnosis must be confirmed by isolation of the causative organism. Differentiation from fowl cholera is necessary because of the similarity of the two diseases. The use of mice and pigeons for inoculation tests is recommended, as is the use of anti-swine-erysipelas serum for neutralization tests in the inoculated test animals. Mice or pigeons given either the pure cultures of the organism or tissues from erysipelas cases die within 21 to 96 hours. Similarly infected animals protected with 0.5 cc. of antiserum do not become sick.

FIG. 41.16 — Erysipelas. Vegetative endocarditis on the tricuspid valves as well as on one of the bicuspid valves in the left side of a turkey's heart. (E. M. Dickinson.)

Another aid to diagnosis is to stain blood or liver smears by Gram's method. The characteristic Gram-positive rods (which decolorize easily, however) are usually grouped in interlacing bundles. The individual organisms are slender, slightly curved, and show characteristic granules. It is possible to isolate the causative organism from the bone marrow of turkeys which have been dead as long as 2 weeks. Jerstad and Dickinson (1956) suggest that because of the persistence of the causative agent in bone marrow, only the shank of a dead bird needs to be shipped to a laboratory for diagnosis.

Prevention, control, and treatment. Since the disease is common in swine and sheep in the United States, turkeys should be kept away from swine and sheep herds in areas where erysipelas is known to exist. It is also unwise to use turkey ranges where the disease has previously occurred. For this reason unnecessary moving of the sick flock to clean range should be avoided.

Grenci (1943) isolated *E. insidiosa* from two samples of fish meal, a common ingredient of turkey feed. Therefore, as a preventive measure, this product should be thoroughly sterilized before being used in turkey feed.

Numerous reports on the use of anti-swine-erysipelas serum and antibiotics have been published. Beaudette and Hudson (1936) found that serum from recovered turkeys protected mice against infection. Lindenmayer and Hamilton (1942) found that 5.0 cc. of formalized serum from sick turkeys had protective value when injected intramuscularly into exposed turkeys. Inconsistent results have been reported on the use of anti-swine-erysipelas serum in sick flocks.

Dickinson (1958) states that treatment of visibly sick birds with penicillin and immediate use of bacterin for all birds is preferable to the use of antiserum.

Recent research on bacterins and antibiotics for prevention and control of the disease in turkeys has yielded encouraging results. Adler and Spencer (1952), Dickinson *et al.* (1953), Moynihan and Stovell (1954), Jerstad and Johns (1954, 1957), and Boyer and Brown (1957a, b) have reviewed the literature on methods of prevention and control and have given their own research results. Jerstad and Dickinson (1956) recommend routine vaccination of flocks of turkeys at 8 to 10 weeks of age in areas where the disease is a yearly problem. The formalized aluminum hydroxide adsorbed bacterin described by Adler and Spencer (1952), Dickinson *et al.* (1953), Jerstad and Johns (1954), and Cooper *et al.* (1957 a, b) is recommended to protect the birds to market age. An initial dose of 2 ml. intramuscularly or subcutaneously is recommended for young birds.

Large toms or breeders can be given 4 ml. booster injections at 16 weeks of age for additional protection. Although intramuscular injections were recommended in the original reports of these investigators, Dickinson (1958) states that intramuscular injections in the breast muscles may result in an inflammatory reaction which may be evident at market time if birds are slaughtered too soon after injection. To avoid this possible reaction, which may cause rejection of carcasses by inspectors, Dickinson suggests that older birds, especially, be injected subcutaneously in the neck about 1 to 2 inches below the skull (Fig. 41.17).

For control of the disease in an outbreak, Jerstad and Dickinson (1956) suggest giving all visibly sick birds penicillin and immediately injecting the entire flock with bacterin. They recommend injection of 0.5 ml. (200,000 units) of aqueous procaine penicillin G or crystalline penicillin G potassium or 1.0 ml. (300,000 units) of aqueous mixture of dibenzylethylenediamine dipenicillin G (150,000 units) and procaine penicillin G (150,000 units). Treatments should be repeated if necessary. Oil diluent preparations are not recommended for advanced cases because of slow action. Also they are difficult to use in cold weather.

Boyer and Brown (1957a, b) reported on the efficacy of oral therapy with antibiotics for control of erysipelas. Procaine penicillin in drinking water at 1,200 mg. per gallon, or in feed at 600 gm. per ton and aureomycin at 100 gm. per ton of feed plus soluble aureomycin in water at 1,000 mg. per gallon proved effective when used under summer conditions. In winter, slightly larger doses were necessary to get equivalent results due to difference in water and feed intake. These authors concluded that from the standpoint of experimental results and cost procaine penicillin is the most practical and efficient antibiotic to use for erysipelas control.

The existence of different antigenic strains of E. insidiosa as well as the possibility of the occurrence of both avirulent and virulent strains is discussed by Truszczynski (1961). These observations, along with those of Raines and Winkel (1956) that antibiotic resistant strains may exist, emphasize the need for continual examination of strains isolated from field outbreaks in order to give accurate advice on control measures.

McCulloch and Fuller (1941) found that household lye (sodium hydroxide) in dilutions of 1:200 to 1:500 is an effective disinfectant against E. insidiosa. Phenol, liquor cresolis and related disinfectants, tincture of iodine, triethanolammoniumlauryl sulfate, and household soaps were moderately effective, but formalin was ineffective.
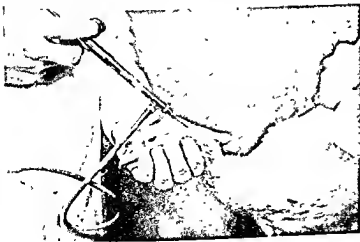
FIG. 41.17 — Older birds should be injected subcutaneously in the neck region as illustrated. (E. M. Dickinson.)

Caution. Since the causative organism of this disease is pathogenic for man, extreme care should be taken when handling infected birds or tissues. Erysipeloid cases caused by contact with diseased turkeys have been reported by Stiles (1916) and Bivins (1919). At least three additional cases (unpublished) have been reported to Hinshaw.

REFERENCES

Adler, H. E., and Spencer, G. R.: 1952. Immunization of turkeys and pigs with erysipelas bacterin. Cornell Vet. 42:238.

Beaudette, F. R., and Hudson, C. B.: 1936. An outbreak of acute swine erysipelas infection in turkeys. Jour. Am. Vet. Med. Assn. 88:475.

Bivins, J. A.: 1949. Erysipelas in turkeys—A case report. Jour. Am. Vet. Med. 114:226.

Boyer, C. I., and Brown, J. A.: 1957a. Studies on erysipelas in turkeys. Avian Dis. 1:42.

——, and Brown, J. A.: 1957b Oral therapy for erysipelas in turkeys. Avian Dis. 1:275.

Coburn, D. R., and Quortrup, E. R.: 1938. Atypical botulism in turkeys. Jour. Am. Vet. Med. Assn. 93:585.

Cooper, M S., Personeus, G. R., and Percival, R. C.: 1957a. Laboratory studies on erysipelas, 4 Duration of immunity in turkeys vaccinated with an adsorbed vaccine. Poultry Sci, 36,266.

——, Personeus, G. R., and Percival, R. C.: 1957b. Laboratory studies on the vaccination of mice and turkeys with an Erysipelothrix rhusiopathiae vaccine. Canad. Jour, Comp. Med. and Vet. Sci. 18:83.

Dickinson, E. M.: 1958 Personal communication

——, Jerstad, A. C., Adler, H. E., Cooper, M., Babcock, W. E., Johns, E. E., and Bottorff, C A.: 1953. The use of Erysipelothrix rhusiopathiae bacterin for the control of erysipelas in turkeys. Proc. 90th Ann. Meet. Am. Vet Med. Assn., p. 370.

Grenci, C. M.: 1948. The isolation of Erysipelothrix rhusiopathiae, and experimental infection of turkeys. Cornell Vet. 38:56.

Hudson, C. B.: 1949 Erysipelothrix rhusiopathiae infection in fowl. Jour. Am. Vet. Med Assn. 115:36.

Jansach, L. W.: 1903. Über die Septikämie der Truthuhner. Oestrer. Monatschr. für Tierheilk. 30:187.

Jerstad, A. C., and Dickinson, E. M.: 1956. Erysipelas of turkeys. Ore. St. Coll., Ext. Bul. 756.

——, and Johns, E. E.: 1954. Performance of a bacterin in the control of erysipelas in turkeys. Proc 91st Ann. Meet. Am. Vet. Med. Assn., p. 333.

——, and Johns, E. E.: 1957. Attempted control of erysipelas in turkeys with furazolidone Jour. Am. Vet. Med. Assn. 130.99.

Lindenmayer, J. E.: 1943. Swine erysipelas in turkeys in the State of Washington. Jour. Am. Vet. Med. Assn. 102.568.

——, and Hamilton, C.M.: 1942. Treatment of swine erysipelas in turkeys with serum from a turkey infected with Erysipelothrix rhusiopathiae. Jour. Am. Vet. Med. Assn. 100.212.

McCulloch, E. C., and Fuller, S. A.: 1941. The relative efficiencies of disinfectants in killing Erysipelothrix rhusiopathiae. Am. Jour. Vet. Res. 2:77.

Moynihan, I. W., and Stovell, P. L.: 1954. The sensitivity of Erysipelothrix rhusiopathiae to antibiotics and its relation to chemotherapy. Proc. 91st Ann. Meet. Am. Vet. Med. Assn., p. 327.

Peterson, E. H., and Hymas, T. A.: 1950. Diamond skin disease (chronic erysipelas) in a turkey. Jour. Am. Vet. Med. Assn. 117:465.

Ralnes, T. V., and Winkel, F. H.: 1956. Erysipelas in pheasants. Jour. Am. Vet. Med. Assn. 129,399.

Rosenwald, A. S, and Dickinson, E. M.: 1941. Swine erysipelas in turkeys. Am. Jour. Vet. Res. 2:202

Stiles, G. W.: 1946. Observation of swine erysipelas in turkeys. (Including the public health aspect and possible human cases.) Jour. Am. Vet. Med. Assn. 109,65.

Truszczynski, M · 1961. The antigenic structure of virulent and avirulent strains of Erysipelothrix rhusiopathiae. Am. Jour. Vet. Res. 22:836.

FOWL CHOLERA*

Fowl cholera, caused by Pasteurella multocida, results in severe economic losses to turkey growers in certain areas. It was first described by DeVolt and Davis (1932), who reported an outbreak in a flock of 175 turkeys in Maryland where there was a 17 per cent mortality. Moynihan and Bankier (1945) have reported the disease in Canada, and Smith and Field (1944) described an outbreak in England. In the writer's experience, the

* See also Chapter 11

disease has been most prevalent in turkeys of about marketable age (6 to 8 months). In many outbreaks chickens have been shown to be the source of the disease, but it may also be carried by adult turkeys.

Signs, course, and mortality. In many respects the clinical signs of fowl cholera resemble those seen in fowl typhoid outbreaks. They include increased thirst, loss of appetite, listlessness, yellow or greenish-yellow, watery diarrhea, and a rise of 2° to 3° F. above normal temperature. The heads appear blue to purplish and have a haggard, drawn appearance. A slimy to gelatinous exudate in the mouth and nostrils is common. The breast muscles become congested, and the skin appears pinkish. Swelling of the snood, similar to that seen in outbreaks of erysipelas (Fig. 41.14) is characteristic in males. Paralysis of the legs and swollen joints are noted in both males and females which develop the chronic type of the disease.

The course of the disease causes acute, heavy losses occurring within a few days, followed by intermittent losses. Symptoms may not be observed before death. In less acute cases the birds linger for several days before dying. Very few sick turkeys recover. Losses vary from a few birds to over half of the flock.

Necropsy findings. The necropsy findings in turkeys are typical of those in chickens, though generally more pronounced. The breast muscles are congested, and the crop usually contains considerable food having a very sour odor. The heart is often enlarged, and the pericardium may be thickened and covered with a whitish-yellow exudate. Petechiae are commonly found over the surface of the pericardial sac, the muscles of the heart, and the adjacent tissues. The pericardial sac may be filled with a yellowish fluid containing whitish-yellow flakes. The liver is enlarged, friable, often salmon colored, and may contain many minute whitish abscesses that give it a mottled appearance. The spleen may be enlarged or show no alteration.

The blood vessels of the mesentery and intestines are usually engorged. The intestines lack tone and often show considerable evidence of hemorrhage, especially in the duodenum. The contents range from a semiliquid to a mucoid consistency. The feces are yellow to yellow-green.

The gizzard seldom contains much food, but the contents present have a peculiar sour odor. The mucous membrane peels readily, and the muscle of the gizzard appears more red than normally. Considerable gelatinous exudate may be present in the proventriculus, and the mucous membrane denuded.

Pneumonia is a characteristic finding. Various stages of lung involvement, from congestion to complete hepatization, are seen. In such cases, the pleural cavity contains a surplus of fluid, or the air sacs may be filled with a semisolid, yellow caseous mass. Similar caseous deposits may be found throughout the abdominal cavity, and such lesions must be differentiated from aspergillosis. In the latter condition the characteristic nodules and "button ulcers" serve as differentiating lesions.

There is a fetid odor to the body cavities and the digestive tract contents of birds suffering from fowl cholera. This odor, difficult to describe, is that of advanced putrefaction and is recognized after experience with a few cases. Isolation of the causative organism is the final means of diagnosis.

Prevention, control, and treatment. Sanitation and hygiene play an important role in prevention. Turkeys should be kept segregated from all fowl that have suffered from the disease. Adult carriers are responsible for the yearly recurrence of the disease on some ranches, and depopulation for a season may be necessary. There is no evidence that the disease is transmitted through the egg, but it is not a good plan to keep for breeding purposes turkeys that have recently suffered from the disease. Complete segregation of the breeding and brooding units or sale of the entire adult flock before any poults are hatched are

recommended as preventive measures on ranches where the disease has existed.

Skidmore's (1932) observations on the common housefly as a possible carrier emphasize the need for keeping turkeys well isolated from chickens or other fowl that might be suffering from the disease, and for prompt destruction of all diseased birds. Wild birds must be considered possible transmitters of the disease. The type of Pasteurella which caused an outbreak studied at the California station (unpublished data) proved to be identical to the one isolated from quail by Hinshaw and Emlen (1943). Burning or the use of a disposal pit instead of burial of dead birds is recommended; otherwise the diseased carcasses, a source of infection, may be dug up by dogs and other animals.

Immunization. Recent researches designed to improve bacterins for use in prevention of fowl cholera have yielded promising results. Pertinent references on this subject include the following: Heddleston and Hall (1958), Heddleston and Reisinger (1959, 1960), Heddleston (1962), Boyer and Brown (1963), and Dorsey (1963a, b).

The current recommendation, based on Heddleston and his co-workers' findings, is to use one of two bacterins. One of these is a water-oil emulsion formalized suspension of *P. multocida* (Heddleston and Reisinger, 1959), and the other is a formalized aluminum hydroxide adsorbed bacterin (Heddleston and Reisinger, 1960). Because of the existence of at least two immunogenic avian types of *P. multocida* (Heddleston, 1962; Dorsey, 1963a), it is recommended that a bivalent bacterin be used. Boyer and Brown (1963) compared bivalent and monovalent bacterins for turkeys and obtained somewhat better results with a bivalent type, but did not get complete protection with either one.

The use of bacterins for prevention of fowl cholera should be confined to those areas where the disease is an economic factor in production. Furthermore, only bacterins which have been proved to be of immunogenic value should be used. Determi-

nation of the immunogenic types of *P. multocida* which are responsible for losses from fowl cholera in a community is suggested. The use of bacterins containing these types will give greater assurance of protection against the disease.

Since much research on the immunization of turkeys is in progress, the reader is urged to continually search the current literature for new advances.

Treatment with drugs. Alberts and Graham (1948) employed sulfamerazine in an experimentally produced outbreak of fowl cholera in turkeys and in a naturally occurring outbreak in adult turkeys. Treated mash containing 0.5 per cent of the drug or 0.5 grain per pound of body weight given orally twice daily prevented losses from the disease during the period of treatment but failed to prevent recurrence of the disease. Their findings suggested sufficient retention of sulfamerazine in the body after discontinuing treatment to suppress *P. avicida* for 2 days after the course of therapy. At the California station sulfamethazine given at the rate of 0.3 per cent in the mash for a period of 3 days definitely reduced losses if given early in an outbreak. The drug did not, however, prevent a recurrence of the disease, nor were carriers eliminated. No evidence of immunity was noted following intermittent treatments over a period of several weeks. Sulfamethazine in amounts greater than 0.3 per cent affected egg quality in laying flocks of turkeys. Peterson (1948) found that 1:2,000 to 1:4,000 dilution of sulfaquinoxaline in drinking water stopped losses in field outbreaks.

McNeil and Hinshaw (1948) found that streptomycin administered in dosages of 150,000 micrograms (25,000 per kilogram of weight) prevented mortality in turkeys artificially infected if treatment was given before or at the same time as inoculation of *P. multocida*. When treatment was delayed 6 to 24 hours or when smaller dosages were given, mortality was prevented, but many chronic cases and carriers developed. Penicillin was ineffective.

Richey and Morgan (1957) found that both chloramphenicol (Chloromycetin) and sulfaquinoxaline reduced losses from fowl cholera if given at the onset of the disease or soon thereafter. Chloramphenicol was given at the rate of 1.0 gm. per pound of feed or 0.2 gm. of chloramphenicol suspension injected intramuscularly. Sulfaquinoxaline was used at the rate of 1.5 gm. per 10 pounds of feed or at the rate of 15 fluid ounces per gallon of water.

REFERENCES

Alberts, J. O., and Graham, R.: 1948. Sulfamerazine in the treatment of fowl cholera in turkeys. Am. Jour. Vet. Res. 9:310.

Boyer, C. I., Jr., and Brown, J. A.: 1963. Research Note- Protection of turkeys vaccinated with fowl cholera bacterins. Avian Dis. 7:165.

DeVolt, H. M., and Davis, C. R.: 1932. A cholera-like disease in turkeys. Cornell Vet. 22.78.

Dorsey, T. A.: 1963a. Studies on fowl cholera. I. A biochemic study of avian *Pasteurella multocida* strains. Avian Dis. 7:386.

——: 1963b. Studies on fowl cholera. II. The correlation between biochemic classification and the serologic and immunologic nature of avian *Pasteurella multocida* strains. Avian Dis. 7:393.

Heddleston, K. L.: 1962. Studies on pasteurellosis. V. Two immunogenic types of *Pasteurella multocida* associated with fowl cholera. Avian Dis. 6:315.

——, and Hall, W. J.: 1958. Studies on pasteurellosis. II. Comparative efficiency of killed vaccines against fowl cholera in chickens. Avian Dis. 2:322.

——, and Reisinger, R. C.: 1959. Studies of pasteurellosis. III. Control of experimental fowl cholera in chickens and turkeys with an emulsified killed vaccine. Avian Dis. 3:397.

——, and Reisinger, R. C.: 1960. Studies on pasteurellosis. IV. Killed fowl cholera vaccine adsorbed on aluminum hydroxide. Avian Dis. 4:429.

Hinshaw, W. R., and Emlen, J. T.: 1943. Fowl cholera in California valley quail. Cornell Vet. 33:331.

McNeil, E., and Hinshaw, W. R.: 1948. The effect of streptomycin on *Pasteurella multocida* in vitro and on fowl cholera in turkeys. Cornell Vet. 38.239.

Moynihan, I. W., and Bankier, J. C.: 1945. An outbreak of fowl cholera in turkeys. Canad. Jour. Comp. Med, and Vet. Sci. 9:46.

Peterson, E. H.: 1948. Sulfonamides in the prophylaxis of experimental fowl cholera. Jour Am. Vet. Med. Assn. 113:263.

Richey, D. J., and Morgan, C. L.: 1957. The effect of chloromycetin and sulfaquinoxaline on fowl cholera in turkeys. Poultry Sci. 36.536.

Skidmore, L. V.: 1932. The transmission of fowl cholera to turkeys by the common housefly (*Musca domestica* Linn.) with brief notes on the viability of fowl cholera microorganisms. Cornell Vet. 22:281.

Smith, H. W., and Field, H. I.: 1944. The isolation of *Pasteurella aviseptica* from a turkey. Vet. Jour. 100.35.

FOWL POX[*]

Fowl pox, a disease of the unfeathered parts of the birds' bodies, is characterized by the formation of pustules and scablike processes. It is caused by a filterable virus, pathogenic for chickens as well. Brunett (1933) tested fowl pox viruses of chicken, turkey, and pigeon origins and reported that the turkey strain was pathogenic for chickens, but not for pigeons. All three strains produced lesions in turkeys, but only the chicken and turkey strains produced immunity.

Tietz (1933) reported that turkeys were not susceptible to the strain of pigeon pox viruses used by him. Hinshaw's experience

has been more like that of Tietz, though very slight swellings of the feather follicles have been noted. Coronel (1934), Brandly and Dunlap (1938), and Beaudette and Hudson (1941) published evidence to show that there is a distinct strain of turkey pox virus which differs from both the chicken and pigeon types. Field observations made by Hinshaw suggest that such is the case.

Matheson *et al.* (1931) reported transmission of fowl pox by mosquitoes, and later Brody (1936) published a comprehensive report on subsequent investigations. These investigators found that one species of mosquito (*Aedes aegypti*) was still able to transmit pox 41 days after

* See also Chapter 26.
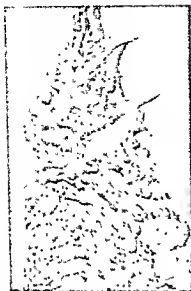
FIG. 41.18—Fowl pox in turkeys. Taken 3 weeks after lesions first appeared. (Hinshaw, Univ. of Calif.)

an initial infective meal. Blanc and Caminopetros (1930) in an earlier paper reported transmission by *Culex pipiens* 58 days after feeding on infected birds.

Signs and necropsy findings. The first indication of pox is the appearance of minute yellowish eruptions on the dewlap, snood, and other head parts. They are soft and, in this pustular stage, easily removed, leaving an inflamed area covered with a sticky serous exudate. The corners of the mouth, the eyelids, and the oral membranes are commonly affected. The lesions enlarge and become covered with a dry scab or a wartlike mass of yellowish-red or brown color (Figs. 41.18 and 41.19). The number of lesions depends on the virulence of the disease. In young poults, the head, legs, and feet may be completely covered with pustules. The disease may even spread to the feathered parts of the body (Fig. 41.20).

Brandly and Dunlap (1938) reported two cases in 3-week-old poults in which the foot pads and foot webs were involved. Large wartlike processes developed which made it difficult for the poults to walk. Except for a lesion of the mouth in one poult, the disease was confined to the feet (Fig. 41.21). In these instances the infection was apparently introduced when the owner "toe punched" the poults for identification purposes.

Males often suffer more than females from the disease, probably because of their inclination for fighting, which spreads the infection through small lacerations.

The mouth parts, the tongue, the esophagus, and occasionally the crop may be covered with masses of soft, yellow cankers closely adhering to the mucous membranes (Figs. 41.19 and 41.22). These yellow, diphtheric ulcers of fowl pox must be differentiated from the small, deep-seated, irregular, diphtheric ulcers or cankers often seen in the mouths of turkeys and not associated with typical head lesions. These cankers are common in turkeys that have been vaccinated against fowl pox or that have recovered from an outbreak. Their cause is not known.

Frequently during the breeding season

FIG. 41.19—Fowl pox lesions in mouth and esophagus. (Hinshaw, Univ. of Calif.)

atypical cases of fowl pox appear in adult turkeys which have been vaccinated with chicken pox vaccine several months previous In these outbreaks, which usually involve only a small percentage of the birds, the mucous membranes of the eyes and the mouth are the principal parts affected. Externally, no lesions in the eyes may be visible, but when the inner surfaces of the lids are examined, soft yellow diphtheric ulcers will be found to be the cause of the increased lacrimation and inflammation of the eye. Typical yellow cankers described above characterize the mouth lesions. There are no internal lesions that are characteristic of the disease.

FIG. 41.20 — Fowl pox lesions on the skin of breast of an adult turkey hen. The head parts were also severely affected. (Hinshow, Univ. of Calif.)

FIG. 41.21 — Fowl pox lesions in the foot pads accidentally introduced during toe marking operations. (Dept. of Anim. Path. and Hyg., Univ. of Ill.)
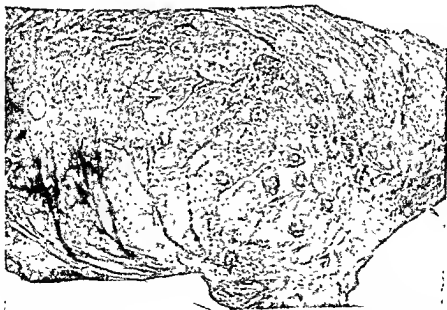
FIG. 41.22 — Fowl pox lesions in the mucous membrane of the crop of a turkey. Lesions covered the head and extended from the mouth to the crop in this bird. (Hinshow, Univ. of Calif.)

The finding by Pilchard *et al.* (1962) that serum from turkeys hyperimmunized against fowl pox virus show neutralizing activity may prove of value in differential diagnosis. It is of interest also that they were able to demonstrate the presence of fowl pox virus (viremia) in the serum of the inoculated turkeys for at least two weeks after disappearance of visible skin lesions.

Ophthalmitis caused by *Aspergillus fumigatus* (Moore, 1953) and keratoconjunctivitis described by Bierer (1956) must be differentiated from ophthalmitis caused by fowl pox virus.

Course and mortality. There is a marked difference in the severity of cases of fowl pox in turkeys and, consequently, variations in the course of the disease. Whereas mild cases may clear up in 2 or 3 weeks, severe outbreaks often last for 6, 7, or even 8 weeks. The canker or mouth types take longer to clear up. In such cases, starvation is the cause of death. Blindness often occurs after the closing of the eyes by severe infection of the eyelids. When the eye is involved, a yellowish cankerlike lesion develops on the mucous membrane of the lid.

The flock mortality is usually low, most of the losses being caused by blindness or starvation. Setback in development and loss in weight are of greater financial importance in the growing flock than the loss by deaths. As outbreaks commonly occur a few days or weeks before market time, it is often necessary to postpone killing the birds for several weeks. If the flock escapes an outbreak before market time, the disease sometimes appears in the breeding birds and causes severe losses through lowered egg production and poor fertility.

Prevention. Vaccination with live-virus vaccine, together with the usual sanitary program, is the recommended method for preventing fowl pox in turkeys. *The problem differs from that in chickens* because in the latter the effect of the disease and vaccination on egg production must be considered in the preventive program, while in the former a meat-producing bird only is involved. Healthy turkeys respond to vaccination, when virus of chicken origin is used, with little or no systemic disturbance such as sometimes follows vaccination of chickens. Vaccines made from turkey strains of virus cause more severe reactions than those made from chicken types, but the duration of immunity is no greater. Pigeon type vaccines produce little or no immunity in turkeys and should not be used.

Attention is called to the possibility of stimulation of parthenogenesis in turkey eggs following the use of live-virus fowl pox vaccines (Olsen, 1956, 1962; and Olsen and Poole, 1962).

Need for vaccination. Fowl pox is so widespread in most turkey-growing areas that yearly vaccination of all turkey flocks is good insurance.

The disease is probably carried to new areas by mosquitoes, birds, visitors, animals, used feed sacks, and the introduction of new stock. Turkey growers who do not vaccinate should keep a constant watch for the first appearance of lesions and should immediately obtain advice on the best plan of control.

Age for vaccination. The correct age to vaccinate turkeys will depend on the locality. In some areas it has been found necessary to vaccinate them by the end of June, regardless of age, because the prevalence of mosquitoes at that season spreads pox rapidly to all susceptible birds. There are considerable data to indicate that healthy turkeys can be vaccinated at any age. Dunn and Sherwood (1933) have successfully vaccinated day-old turkeys. Many growers have vaccinated successfully at 6 or 8 weeks of age; the majority, however, at 10 to 12 weeks. Extreme care must be taken, when vaccinating young poults, to prevent the vaccine from getting on parts of the body other than the area to be treated. Sometimes a careless operator, after spilling vaccine, holds the poult's head with his contaminated hand. The young, tender skin is so susceptible that a severe case of generalized pox may follow.

As it requires from 4 to 8 weeks for the vaccination lesion (take) to disappear completely, turkeys should be vaccinated at least 8 weeks before market time.

Vaccines of chicken pox origin will produce immunity for about 6 months. It may, therefore, be advisable to revaccinate all birds which are to be kept for breeders 6 or 7 months following the first vaccination. In order to be sure that such birds are no longer immune, it is a good plan to revaccinate a sample (100 birds) of the flock. If after a week the majority of the sample shows takes, the remainder of the flock can be revaccinated. If only a small percentage is susceptible, revaccination of the rest of the flock should be postponed. If birds are revaccinated, it is good practice to use the opposite leg site for the second vaccination in order to avoid possible local skin immunity which would prevent a good reaction.

The wing web is not recommended as a site for applying the vaccine because of disastrous results that have followed the use of this site. One flock of 8-week-old poults under the observation of the writer, which was vaccinated in the web of the wing by the puncture method, suffered nearly a 50 per cent loss from fowl pox. Lesions developed on the head parts, in the mouth, and even in the mucous membrane of the upper esophagus, crop, and lower esophagus. Figure 41.23A and B shows the results of wing-web vaccination in an adult bird. At least 10 per cent of
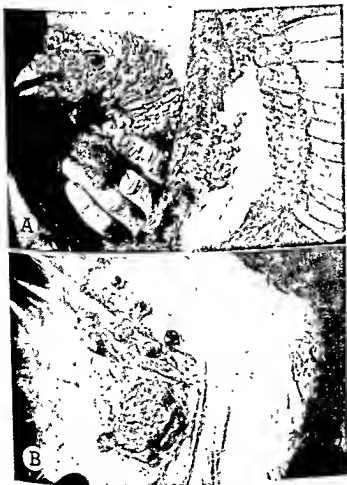
FIG. 41.23 — (A) Wing-web vaccination. Taken about 6 weeks after vaccinating in the wing web by a single stab of the inoculating knife. Shows how the disease spread from this single inoculation to other areas on the wing and head. The bird died within a few days after the picture was taken. (B) Close-up of A at a somewhat earlier stage. (Hinshaw, Univ. of Calif.)

the flock from which the bird came was affected in a similar manner. Such spread of the infection is worse during damp or foggy weather and apparently associated with the birds picking at the vaccinated area before immunity develops.

The skin of the upper thigh has certain advantages over other sites for routine vaccination (Fig. 41.24). These are easy accessibility to the operator, absence of feathers, and inaccessibility to the vaccinated birds or their penmates. The last point is important from the standpoint of the spread of fowl pox by fighting before immunity has been established.

The same methods of applying the vaccine are used for chickens and turkeys, and the reader is referred to the general section on fowl pox for these procedures. Figure 41.28 illustrates a method for handling turkeys while vaccinating them.

Control of an outbreak. If fowl pox appears in a flock, the following procedure is recommended:

1. Isolate all birds showing lesions.
2. Vaccinate as soon as possible all birds not showing lesions.
3. Place infected birds in warm, dry quarters if available.
4. Separate the males or keep careful watch over them to prevent fighting.
5. Treat infected birds individually by removing the scabs and touching the wounds with iodine, iodine ointment, or an antibiotic ointment.
6. Individual feeding of valuable birds with the aid of a funnel and rubber tubing inserted into the crop may be advisable.

Drugs for internal treatment are not

FIG. 41.24 — (A) Restraint of turkey for vaccinating on upper thigh. Note that the table is covered with newspapers. This aids in preventing undue spread of vaccine. (B) A close-up, taken to show the suggested location. The long tuft of feathers that normally covers this naked area is being held back by the vaccinator's left hand. The heavy glass inkwell is a convenient holder for the vaccine. To prevent excessive dust contamination, it is covered with a piece of rubber with a small opening. (Hinshaw, Univ. of Calif.)

recommended. Since loss of flesh and retarded development are the chief causes of economic loss in most outbreaks, careful management and attention to the feeding program during and after an outbreak are essential for a speedy return to normal. The course of the disease can be shortened by the use of shelter for roosting and by general protection from damp weather.

REFERENCES

Beaudette, F. R., and Hudson, C. B.: 1941. Egg propagation of turkey pox virus. Poultry Sci. 20:79.

Bierer, B. W.: 1956. Keratoconjunctivitis in turkeys—a preliminary report. Vet. Med. 51:363.

Blanc, G. and Caminopetros, J.: 1930. La transmission des varioles aviaires par les moustiques. Acad. Sci. (Paris) Compt. Rend. 190:954.

Brandly, C. A., and Dunlap, C. L.: 1938. An outbreak of pox in turkeys, with notes on diagnosis and immunization. Poultry Sci. 17:511.

Brody, A. L.: 1936. The transmission of fowl-pox. Cornell Univ. Agr. Exper. Sta., Memoir 195:1.

Bruneta, E. L.: 1933. Some observations on pox virus obtained from a turkey N.Y. St. Vet. Coll. Ann. Rep. 1932-33, p. 69.

Coronel, A. B.: 1934. Fowl-pox vaccine from virus of turkey origin. Philippine Jour. Anim. Ind. 1:85.

Dunn, R. C., and Sherwood, R. M.: 1933. Immunization of day-old chicks and poults against fowl pox. Poultry Sci. 12:323.

Matheson, R., Brunett, E. L., and Brody, A. L.: 1931. The transmission of fowl-pox by mosquitoes (preliminary report). Poultry Sci. 10:211.

Moore, E. N.: 1953. Aspergillus fumigatus as a cause of ophthalmitis in turkeys. Poultry Sci. 32:796.

Olsen, M. W.: 1956. Fowl pox vaccine associated with parthenogenesis in chicken and turkey eggs. Science 124:1078.

——: 1962. Killed-virus vaccines in relation to parthenogenetic development in turkey eggs. Am. Jour. Vet. Res. 23:855.

——, and Poole, H. K.: 1962. Further evidence of a relationship between live fowl pox virus and parthenogenesis in turkey eggs. Proc. Soc. Exp. Biol. Med. 109:944.

Pilchard, E. I., Jr., Hanson, L. E., and Alberts, J. O.: 1962. Fowlpox virus neutralization antibody and viremia in turkeys. Avian Dis. 6:596.

Tietz, G.: 1953. Ueber die Empfänglichkeit verschiedener Vogelarten für eine Infektion mit originärem Huhner- und Taubenpockenvirus Arch f Tierheilk. 65 244.

INFECTIOUS SINUSITIS (Swellhead, Air-Sac Infection, Chronic Respiratory Disease—CRD, Airsacculitis, Mycoplasmosis)

This disease is of great economic importance wherever turkeys are reared. It is characterized by inflammation of the lining membranes of the infraorbital sinuses, following which the sinuses become distended with a semigelatinous exudate (Fig. 41.25 A and B). Lower respiratory tract involvement is also seen, with the air sacs the most usual site of infection. The period of incubation is erratic and may vary from 1 week to several weeks. The first accurate description of the disease was probably made by Dodd (1905) in England under the name of "epizootic pneumoenteritis." M'Fadyean (1893) had already reported a similar disease in England which he called "pneumo-endocarditis" and which may have been the same as

that described by Dodd. In the United States, Tyzzer (1926) was the first to describe it. The name "infectious sinusitis" was suggested by Dickinson and Hinshaw (1938) to differentiate it from the inflammation of the sinuses noted in vitamin A deficient turkeys.

Excellent reviews on the disease comparing it to the similar disease CRD in chickens are given by Van Roekel et al. (1957) and Osborn and Pomeroy (1958a). In September, 1962, the U.S. Department of Agriculture sponsored a workshop on Mycoplasmosis as it affects both chickens and turkeys. The report of this group (Anon., 1962) summarizes the current status of the disease and makes specific recommendations on diagnosis, handling, and preventing outbreaks. Since it is generally accepted that chronic respiratory disease (CRD) of chickens and infectious sinusitis of turkeys are etiologically similar, the

reader is referred to Chapter 13 for a detailed review.

Etiology. It is generally accepted that a pleuropneumonialike organism, *Mycoplasma gallisepticum*, is an important etiological factor, if not the specific causative agent, of infectious sinusitis. A number of investigators (Jerstad and Hamilton, 1948; Groupé and Winn, 1949; Delaplane, 1919; Jerstad et al., 1950; Van Roekel et al., 1952; Grumbles et al., 1952) showed the relationship of the two diseases before Markham and Wong (1952) succeeded in propagating the agents on a cell-free medium and identifying them.

Evidence is accumulating that there are multiple antigenic types of Mycoplasma prevalent in both turkeys and chickens, and that strains vary in their ability to produce disease (Adler et al., 1957; Grumbles et al., 1958; Jerstad et al., 1959c; Adler, 1960; Kleckner, 1960; and Yoder and Hofstad, 1962). Evidence that *M. gallisepticum* in pure culture can produce the respiratory phase of the disease in "germ-free" turkeys has been reported by Smibert et al. (1959a). Winterfield (1953) describes a case in pigeons which was transmitted to turkeys and in which typical sinusitis developed.

Van Roekel et al. (1957), in a technical report on research on the etiology and pathology of CRD in chickens, include a good historical review on the etiology of infectious sinusitis in turkeys.

Signs. Two forms of the disease are recognized. The sinus form is the one from which the disease derives its name. The other is the respiratory form which involves the lower respiratory tract and especially the air sacs and produces a syndrome resembling CRD in chickens. Prodromes of the disease are given when birds shake their heads and when discharges are found on the feathers over the wing where the bird has attempted to clean its nostrils. These manifestations are followed by foaming of the eye secretions and by a clear nasal discharge. Swelling of the sinuses and, in advanced cases, a partial to complete closing of the eyes follow these early signs (Fig. 41.25). The appetite remains good as long as the bird can see to eat. As the disease progresses, the affected birds become thin. Labored breathing, in some cases, results from air-sac involvement or from complete closing of the palatine opening because of pressure from the exudate in the sinuses.
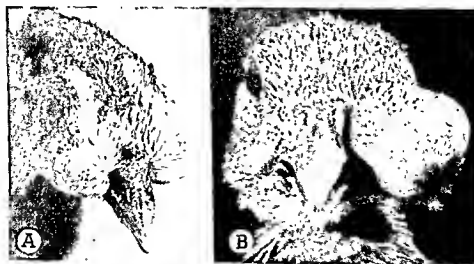
FIG. 41.25 — (A) An advanced case of infectious sinusitis involving both sides of the face. (B) A similar case after the exudate in one sinus has been removed. (Hinshaw, Univ. of Calif.)

Bacteria may cause similar clinical signs. Beach and Schalm (1936) produced typical sinusitis in turkeys with *Hemophilus gallinarum*, the causative organism of fowl coryza. Delaplane (1944) isolated a Pasteurellalike bacterium from exudate in field cases of sinusitis in turkeys. This organism produced typical signs in chickens, and exudate from the infected sinuses of the chickens produced sinusitis in turkeys. The role which other microorganisms, including other *Mycoplasma* species, play in production of similar signs and lesions is reported by Smibert et al. (1959b) and Kumar et al. (1963).

Fahey (1956) described a chronic respiratory disease in turkeys from which he isolated both a virus and a PPLO. In the outbreaks described by him, 2 weeks after onset of respiratory symptoms, 40 to 60 per cent of the birds were manifesting sinusitis. A review of the current status of the role of this virus as a cause of respiratory disease in turkeys is reported by Subramanyam and Pomeroy (1960).

The disease must also be differentiated from sinusitis associated with vitamin A deficiency. Mechanical injury caused by a piece of grain or mash or other foreign body becoming lodged in the sinus may result in a swollen sinus. As a rule these mechanical cases are unilateral.

Transmission. Jerstad et al. (1950) were able to transmit the disease experimentally by instillation of infective material by several routes. These included drop instillation into the palatine cleft of 2- and 3-day-old poults, instillation into the crop, intramuscular injection, swabbing the trachea, and by contact with birds manifesting the respiratory form of the disease, as well as by the usual procedure of injecting the infective material into the infraorbital sinuses. They were able to confirm Jungherr's (1949) contention that the two common manifestations of the disease—swollen sinuses and air-sac infection—are one and the same entity. The incubation period varied from 2 weeks to 17 weeks. Field observations indicate the disease is airborne and that dust storms play an impor-

tant part in transmission. Air-borne transmission of the disease is more likely to occur, according to the writer's experience, if the lower respiratory form is prevalent.

There is ample evidence to indicate that in certain stages, the disease may be transmitted in a small percentage of the eggs laid by infected birds. Jerstad et al (1949 and 1959a, b) demonstrated that transmission of the disease is possible, but somewhat inconsistent. They concluded by experimental as well as by field observations that infected breeders may transmit the disease to some of their progeny. They found no evidence that so-called silent carriers in a healthy flock are at important factor in egg transmissions. Hofstad (1957a), Richey et al. (1958). Osborn and Pomeroy (1958b), Abbott et al. (1960), and Kumar et al. (1963) have reported additional evidence of egg transmission.

Course and Mortality. Sinusitis of the contagious type runs a chronic course and may exist in a flock for weeks. Although the number of deaths may be less than in some more acute diseases, the financial loss may be greater. Failure to gain weight accounts for as much damage as does mortality.

Necropsy findings. The filling of the sinuses with exudate, the presence of pneumonia, and pleuritis are manifestations of sinusitis. This disease may occur without involvement of the other respiratory passages, but inflammatory changes in all the respiratory organs may be noted. In some instances, the lesions will be confined to the lower respiratory passages without involvement of the sinuses. Caseated exudate in the air sacs is common in acute outbreaks. When the lungs are affected, the bronchi are chiefly concerned.

The exudate in the sinuses in the first stages is watery in consistency, later becoming semigelatinous and finally caseated and whitish-yellow in color. In typical outbreaks, caseation of the exudate is the exception. In sinusitis associated with vitamin A deficiency, the lesions described under avitaminosis A will also be seen.

References on the microscopic pathology

of the disease include Jungherr (1949), Hitchner (1949a), and Barber (1962). Jungherr reported that histopathologically experimental sinus infection was characterized in the early stages by severe catarrhal inflammation of the mucosa and in the later stages by infiltration, fibrosis, and hyperplastic lymphofollicular nodules in the submucosa. Pneumonia and air-sac infections were accompanied by development of massive lymphofollicular nodules which assumed a pathognomonic significance. Cordy and Adler (1957) describe an encephalitis produced artificially in turkeys by a neurotropic strain of PPLO isolated from a naturally occurring case of infectious sinusitis which had exhibited symptoms of encephalitis.

Final diagnosis depends on isolation and identification of the causative agent.

Prevention, control, and treatment. Turkeys which recover from sinusitis may remain carriers the following year, so all contact between them and growing poults should be avoided.

Since egg transmission is possible, every effort should be made to eliminate from breeding programs those flocks that have a history of previous infections.

The recent findings that the causative agent of chronic respiratory disease of chickens will cause the disease presents another reason for keeping turkeys segregated from chickens.

It has not been possible to artificially immunize turkeys against the disease (Adler *et al.*, 1960). Recovery from the disease according to Adler and his associates does confer a measurable degree of immunity but does not eliminate the carrier state of all recovered birds.

Detection of carriers is possible by means of the hemagglutination inhibition (HI), tube agglutination, and slide agglutination tests using *M. gallisepticum* (S-6) antigen (Hofstad, 1957b; Adler, 1958; and Adler *et al.*, 1962). Although the slide agglutination test is less sensitive than the tube agglutination test, Adler *et al.* (1962) stated that with an improved antigen they have found it suitable for routine testing. They cautioned that an antigen satisfactory for testing chicken sera might be unsatisfactory for testing turkey sera. These serologic tests, according to Adler *et al.*, are valuable to detect flock infections and the stage of disease in a flock but not in individuals. They are not recommended for use in repeated tests for removal of reactors to eliminate the disease from a breeding flock. They might be used in selecting the market time for birds that have gone through an acute stage of the disease. Both Hofstad (1957b) and Adler (1958) showed a reduction in the serologic titer following medication. This suggests another possible application of the tests.

A number of the states now have a Mycoplasma infection eradication program which is operated in conjunction with the pullorum disease-fowl typhoid program in cooperation with the National Poultry and Turkey Improvement Plans. (For details the reader is referred to his state official agency for these plans.) Elimination of infected flocks and the establishment of Mycoplasma-free breeding flocks as sources of replacement flocks are the primary objectives of these cooperative plans. The general practices and recommendations used by them are outlined in the U.S.D.A. Committee Report on *Mycoplasma gallisepticum* Inspection in Poultry (Anon., 1962). A recent publication by Rosenwald and Adler (1962) is also recommended.

Madsen (1938) reported good results in the control of sinusitis, uncomplicated by lower respiratory involvement, by the use of 1.0 cc. of a 4 per cent solution of silver nitrate injected into the affected sinus after the removal of the sinus exudate with the aid of a hypodermic syringe. Tyzzer (1926), Dickinson and Hinshaw (1938), and Hart (1940) have used a 15 per cent argyrol solution in a similar manner. McNeil and Hinshaw (1946) compared the efficiency of silver nitrate with three colloidal silver preparations and two ephedrine remedies containing sulfonamides. The latter were of no value, while the colloidal silver drugs were from 50 to 70 per cent effective as compared with

about 85 per cent effectiveness of the silver nitrate. *These silver preparations have little if any value in flocks where the majority of the birds are suffering from air-sac infection as well as swollen sinuses.*

The method, which can also be used for injection of antibiotics or other drugs, consists in withdrawing the gelatinous exudate from the sinus with the aid of a 5 or 10 cc. syringe fitted with a 15- or 16-gauge needle, 1½ inches long. The needle is inserted through the skin and sinus membranes into the filled sinus. Withdrawal of the syringe plunger will remove the semifluid exudate. The needle is left inserted in the sinus, and with a second syringe the remedy is injected and worked through the tissues by gentle massage. Care should be taken to avoid excessive dosage.

These treatments cause considerable swelling of the affected areas, but this subsides within 2 or 3 days, and complete recovery usually takes place within 10 days. In severe cases a second treatment may be necessary.

It is essential to administer this treatment in the early stages of the disease when the exudate is in a semigelatinous state. Figure 41.26 shows the method of inserting the needle for removal of the exudate, and for injection of the remedy. If silver nitrate solution is used, it should be freshly prepared, and it is advisable for

the operator to use leather or rubber gloves because this remedy is caustic to the skin.

Of the antibiotics tried, streptomycin, erythromycin, Tylosin, and chloramphenicol appear to be the ones of choice for injection into infected sinuses. Reports on the effectiveness of streptomycin have been published by Groupé and Winn (1949), Hitchner (1949b), McArthur (1950), Glover (1950), and Grumbles and Boney (1951). Hitchner found that 0.6 ml. of a sterile aqueous solution containing 150 mg. of the drug, injected directly into the sinuses without removal of exudate, resulted in a high percentage of recoveries in 7 to 14 days. The effective dose of Aureomycin given by Hitchner is from 25 to 50 mg. Hamdy *et al.* (1958), Holper *et al.* (1958), and Barnes *et al.* (1960) all found erythromycin to be effective against the sinusitis form if given intrasinally at the rate of 50 mg. per sinus after the removal of the exudate. Intramuscular injections or the use as a feed additive were both ineffective. A review on the use of Tylosin is given by Yoder *et al.* (1961).

In England, Cook *et al.* (1963) have reported on the effective use of Spiramycin in the treatment of mycoplasmosis of turkeys. Cure rates up to 90 per cent were reported following the subcutaneous injection of single doses of 100 mg. per kilogram of body weight. The recommended site of
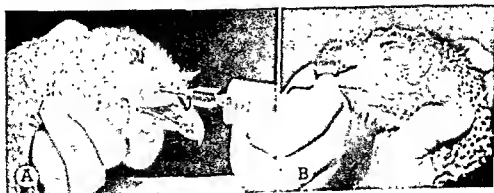
FIG. 41.26 — (A) Treatment of infectious sinusitis. Method of insertion of the hypodermic needle into the sinus for withdrawal of exudate and inoculation of remedy. (B) After withdrawal of the exudate, the needle is left in the sinus until the therapeutic agent is introduced. (Hinshaw, Univ. of Calif.)

inoculation was the area at the base of the neck.

The information available on the effectiveness of these drugs for the lower respiratory type of the disease indicates that the broad-spectrum antibiotics are of value. Hitchner injected 150 to 250 mg of streptomycin into the dewlaps of five turkeys showing symptoms of only the lower respiratory tract type. Four of these became free of clinical signs within 8 days and the fifth in 15 days following treatment. Injection of the drug into the dewlap had no effect on infected sinuses. Grumbles and Boney (1950) reported little or no effect on the lower respiratory disease following treatment with streptomycin.

Grumbles and Boney (1951) used chloramphenicol and Terramycin in the feed for turkeys suffering from both the sinusitis and lower respiratory types of the disease. Chloramphenicol in an all-mash ration was effective in treatment of both types when given at levels of 0.25 to 0.5 per cent in the feed for 8 to 12 days. Terramycin was not as effective in the few birds tried.

Benton and Cover (1958) compared the effectiveness of injections of several of the nitrofurans, two antibiotics, and silver nitrate in treatment of experimentally

produced sinusitis. The nitrofurans were also used in drinking water and applied as an aerosol (dusting). Results obtained were not encouraging. Drugs injected directly into the sinuses were more effective than those used in drinking water or as an aerosol.

Osborn and Pomeroy (1958b) found that prolonged high-level treatment of turkey hens with broad-spectrum antibiotics did not completely eliminate egg transmission. Drug-resistant strains may complicate any control program (Fahey, 1957).

The use of antibiotics for elimination of *Mycoplasma* sp. from hatching eggs as an aid in preventing spread by this means has been reported by a number of investigators including Chalquist and Fabricant (1959), Levine and Fabricant (1962), and Olson *et al.* (1962). The method consists in dipping warmed (37° C.) eggs into a chilled solution of the antibiotic in an attempt to produce sufficient absorption of the antibiotic from the dip solutions into the eggs to prevent infection of the developing embryo. Oxytetracycline, erythromycin, and Tylosin have yielded encouraging results in the limited experimental trials reported. The practicability of this method of destroying microorganisms in hatching eggs is yet to be determined.

REFERENCES

Abbott, U. K., McMartin, D. A., Adler, H. E., and Kratzer, F. H.: 1960. The effect of egg-borne Mycoplasma on embryonating turkey eggs. Poultry Sci. 39:315.

Adler, H. E.: 1958. A PPLO slide agglutination test for the detection of sinusitis of turkeys. Poultry Sci. 37.1116.

———: 1960. Mycoplasma, the cause of chronic respiratory disease. *In* Biology of the Pleuropneumonialike Organisms. Annals N.Y. Acad. Sci. 79(10):703.

———, McMartin, D., and Shifrine, M.: 1960. Immunization against Mycoplasma infections of poultry. Am. Jour. Vet. Res. 21:482.

———, Shifrine, M., and Ortmayer, H.: 1962. Interpretation of *Mycoplasma gallisepticum* serologic tests for turkeys. Proc. Twelfth World's Poultry Cong. Sydney, Australia. 1962, p. 322.

———, Yamamoto, R., and Berg, J.: 1957. Strain differences of pleuropneumonia-like organisms of avian origin. Avian Dis. 1:19.

Anonymous: 1962. A committee report on *Mycoplasma gallisepticum* infection in poultry. USDA, ARS 22 81. 17 pp.

Barber, C. W.: 1962. The lymphofollicular nodules in turkey tissues associated with *Mycoplasma gallisepticum* infection. Avian Dis. 6.289.

Barnes, L. E., Ose, E. E., and Gossett, F. O.: 1960. Treatment of experimental infectious sinusitis of turkeys with erythromycin. Avian Dis. 4:176.

Beach, J. R., and Schalm, O. W.: 1936. Studies of the clinical manifestations and transmissibility of infectious coryza of chickens. Poultry Sci. 15.466.

Benton, W. J., and Cover, M. S.: 1958. Experimental treatment of infectious sinusitis in turkeys with nitrofurans and antibiotics. Am. Jour. Vet. Res. 19:489.

Chalquest, R. R., and Fabricant, J.: 1959. Survival of PPLO injected into eggs previously dipped in antibiotic solutions. Avian Dis. 3:257.

Cook, J., K. A., Inglis, J. M., and Parker, W. G. C.: 1963. Spiramycin adipate in the treatment of mycoplasmosis in turkeys. Vet. Record 75.215.

Cordy, D. R., and Adler, H. E.: 1957. The pathogenesis of the encephalitis in turkey poults produced by a neurotropic pleuropneumonia-like organism. Avian Dis. 1:235.

Corstvet, R. E., and Sadler, W. W.: 1964. The diagnosis of certain avian diseases with the fluorescent antibody technique. Poultry Sci. 43:1280.

Delaplane, J. P.: 1944. A Pasteurella or Pasteurella-like organisms as the cause of an infectious sinusitis of turkeys. Poultry Sci. 23:247.

———: 1949. Cultivation of the chronic respiratory disease virus in chick embryos. Proc. 53rd Ann. Meet. U.S. Livestock Sanit. Assn., p. 193.

Dickinson, E. M., and Hinshaw, W. R.: 1938. Treatment of infectious sinusitis of turkeys with argyrol and silver nitrate. Jour. Am. Vet. Med. Assn. 93:151.

Dodd, S.: 1905. Epizootic pneumo-enteritis of the turkey. Jour. Comp. Path. and Therap. 18.239.

Fahey, J. E.: 1956. A virus in chronic respiratory disease of turkeys. Nature 177:90.

———: 1957. Infectious sinusitis of turkeys caused by antibiotic resistant pleuropneumonia-like organisms. Vet. Med. 52:305.

———, and Crawley, J. F.: 1954. Studies on chronic respiratory disease. II. Isolation of the virus. Canad. Jour. Comp. Med. 18:13.

Glover, J. S.: 1950. Report of treatment of infectious sinusitis of turkeys with streptomycin. Canad. Jour. Comp. Med. and Vet. Sci. 14:166.

Groupé, V., and Winn, J. D.: 1949. The characteristics of an agent morphologically resembling the *Chlamydozoaceae* and causing sinusitis in turkeys. Jour. Bact. 57:515.

Grumbles, L. C., and Boney, W. A.: 1950. Infectious sinusitis of turkeys. Proc. 54th Ann. Meet. U.S. Livestock Sanit. Assn., p. 166.

——— and Boney, W. A.: 1951. *Chloromycetin and terramycin for infectious sinusitis of* turkeys. Jour. Am. Vet. Med. Assn. 119.384.

———, Boney, W. A., and Delaplane, J. P.: 1952. The spread of infectious sinusitis of turkeys to chickens by natural means. Poultry Sci. 31:809.

———, Flowers, A. I., and Cassidy, D. R.: 1953. The role of pleuropneumonia-like organisms in infectious sinusitis of turkeys. Avian Dis. 2:336.

Hamdy, A. H., Sanger, V. L., and Gale, C.: 1958. The use of erythromycin in the treatment of infectious turkey sinusitis during a natural outbreak. Avian Dis. 2:250

Hart, J.: 1910. Sinusitis in turkeys. Australian Vet. Jour. 16:163.

Hitchner, S. B.: 1949a. The pathology of infectious sinusitis of turkeys. Poultry Sci. 28:106.

———: 1949b. Streptomycin as a treatment for infectious sinusitis of turkeys. Poultry Sci. 28.627

Hofstad, M. S.: 1957a. Egg transmission of infectious sinusitis in turkeys. Avian Dis. 1:165.

———: 1957b. A serological study of infectious sinusitis of turkeys. Avian Dis. 1:170.

Holper, J. C., Bower, R. R., and Sylvester, J. C.: 1958. The effect of erythromycin on experimental infectious sinusitis of turkeys. Avian Dis. 2.290.

Jerstad, A. C.: 1964. Isolation of *Mycoplasma gallisepticum* from fresh eggs and blood. Avian Dis. 8:56.

———, and Hamilton, C. M.: 1948. The etiology of infectious sinusitis of turkeys. Poultry Sci. 27:609.

———, Hamilton, C. M., and Peterson, E. H.: 1950. Experimental transmission of infectious sinusitis of turkeys. Am. Jour. Vet. Res. 11:260.

———, Hamilton, C. M., and Smith, V. E.: 1949. Preliminary report of probable egg transmission of infectious sinusitis. Vet. Med. 44 272.

———, Hamilton, C. M., and Smith, V. E.: 1959a. Egg transmission of infectious sinusitis in naturally infected turkeys. Avian Dis. 3:28.

———, Hamilton, C. M., and Smith, V. E.: 1959b. Egg transmission of infectious sinusitis following inoculation of turkey breeders in egg production. Avian Dis. 3.105.

———, Hamilton, C. M., and Smith, V. E.: 1959c. The clinical course of infectious sinusitis in experimentally infected turkeys. Avian Dis. 3:114.

Jungherr, E. L.: 1949. The pathology of experimental sinusitis of turkeys. Am. Jour. Vet. Res. 10.372.

Kleckner, A. L.: 1960. Serotypes of avian pleuropneumonia-like organisms. Am. Jour. Vet. Res. 21:274.

Kumar, S., Dietks, R. E., Newman, J. A., Pfow, C. J., and Pomeroy, B. S.: 1963. Airsacculitis in turkeys. I. A study of airsacculitis in day-old poults. Avian Dis. 7.376.

Levine, P. P., and Fabricant, J.: 1962. Effect of dipping eggs in antibiotic solutions on PPLO transmission in chickens. Avian Dis. 6 72.

McArthur, F. X.: 1950. Streptomycin in infectious sinusitis. Jour. Am. Vet. Med. Assn. 116 250

M'Fadrean, J.: 1898. Epizootic pneumo-enteritis in the turkey. Jour. Comp. Path. and Therap 6.334.

McNeil, E., and Hinshaw, W. R.: 1946. Recent studies on the treatment of infectious sinusitis in turkeys. Jour. Am. Vet. Med. Assn. 108.260.

Madsen, D. E.: 1938. Sinusitis of turkeys and its treatment. Utah Agr. Exper. Sta., Bul. 280.

Markham, F. S., and Wong, S. C.: 1952. Pleuropneumonia-like organisms in the etiology of turkey sinusitis, and chronic respiratory disease of chickens. Poultry Sci. 31:902.

Noel, J. K., DeVolt, H. M., and Faber, J. E.: 1964. Identification of *Mycoplasma gallisepticum* in lesion tissue by immunofluorescence. Poultry Sci. 43:445.

Olson, H. R., and Flint, J. G.: 1964. State-wide eradication of PPLO infection in turkeys. Scientific proceedings, 101st Ann. Meet. Am. Vet. Med. Assn., p. 292.

Olson, N. O., Hash, T. R., Heishman, J. O., and Campbell, A.: 1962. Dipping of hatching eggs in erythromycin for the control of Mycoplasma. Avian Dis. 6:191.

Osborn, O. H., and Pomeroy, B. S.: 1958a. Symposium on chronic respiratory diseases of poultry. V. Infectious sinusitis of turkeys. Am. Jour. Vet. Res. 19:468.

———, and Pomeroy, B. S.: 1958b. The effect of antibiotics on the infectious sinusitis agent of turkeys. Part I. Egg transmission. Avian Dis. 2:180

Rhoades, K. R., Kelton, W. H., and Heddleston, K. L.: 1964. Experimentally induced mycoplasmosis in adult turkeys. Am. Jour. Vet. Res. 25:764.

Richey, D. J., Barnett, B. D., Boone, M. A., and Morgan, C. L.: 1958. Transmission and control of infectious sinusitis in turkeys. Avian Dis. 2:173.

Rosenwald, A. S., and Adler, H. E.: 1962. Controlling infectious sinusitis of turkeys. Calif. Agr. Exper. Sta. Ext. Ser. Cir. 507. 19 pp.

Sadler, W. W., Corstvet, R. E., and Adler, H. E.: 1961. The effect of *Mycoplasma gallisepticum* infection on the wholesomeness of market turkeys. Avian Dis. 8.596

Smibert, R. M., Forbes, M., Faber, J. E., Gabuten, A. R., and DeVolt, H. M.: 1959a. Studies on "air-sac" infection in poultry. 1. Infection of germ-free turkeys with *Mycoplasma gallinarum* (Avian PPLO) from sinal exudate and broth cultures. Poultry Sci. 38:676.

———, Faber, J. E., and DeVolt, H. M.: 1959b. Studies on "air-sac" infection in poultry. 2. Bacterial flora of the respiratory system of turkeys associated with avian PPLO (Pleuropneumonia-like organisms) in artificially induced aerosaccitis. Poultry Sci. 38:1598.

Subramanyam, P., and Pomeroy, B. S.: 1960. Studies on the Fahey Crawley virus. Avian Dis. 4:165.

Tyzzer, E. E.: 1926. The injection of argyrol for the treatment of sinusitis in turkeys. Cornell Vet. 16.221.

Van Roekel, H., Gray, J. E., Shipkowitz, N. L., Clarke, M. K., and Luchini, R. N.: 1957. Etiology and pathology of the chronic respiratory disease in chickens. Mass. Agr. Exper. Sta., Bul. 486.

———, Olesiuk, O. M., and Peck, H. A.: 1952. Chronic respiratory disease of chickens. Am. Jour. Vet. Res. 13.252

Winterfield, R. W.: 1955. Pigeons as a source of the infectious sinusitis agent. Vet. Med. 48:124.

Yamamoto, R., Babcock, W. E., and Dickinson, E. M.: 1965 Antibody response of turkeys exposed to *Mycoplasma gallisepticum*. Proc. 67th Ann. Meet. U.S. Livestock Sanit. Assn., p. 578.

Yoder, H. W., Nelson, G. L., and Hofstad, M. S.: 1961. Tylosin an effective antibiotic for treatment of PPLO sinusitis. Vet. Med. 56:178.

———, and Hofstad, M. S.: 1962. A previously unreported serotype of avian Mycoplasma. Avian Dis. 6 147

———, and Hofstad, M. S. 1964. Characterization of avian Mycoplasma. Avian Dis. 8:481.

FOWL TYPHOID[*]

Fowl typhoid is a septicemic infection caused by *Salmonella gallinarum*. Contact with chickens or yards used by chickens is an important factor in the spread of fowl typhoid to turkeys. Pfeiler and Roepke (1917), Kaupp and Dearstyne (1924), Martinaglia (1929), and Hinshaw (1930) reported the disease in turkeys reared on farms where it was also prevalent in chickens. Evidence that the disease may be transmitted through the egg in the same manner as is pullorum disease is presented by Boney (1947), Hinshaw and Taylor (1933), and Johnson and Pollard (1940). Johnson and Pollard reported outbreaks in poults with clinical signs, mortal-

ity, and pathology comparable to those of pullorum disease. Usually, however, the disease is reported in mature or nearly mature turkeys. Vidovic (1931) claimed that strains of the causative organism isolated from turkeys were more pathogenic than strains isolated from chickens. The strains isolated from turkeys by Hinshaw have appeared identical to those isolated from other fowl. A more complete list of references is included in the general section on fowl typhoid.

Signs, course, and mortality. Increased thirst, loss of appetite, listlessness, tendency to separate themselves from the well birds, and greenish to greenish-yellow diarrhea characterize the disease in the field. The sick turkeys sit with drooping tails, sagging wings, and heads hung low or carried back

* See also Chapter 10.

over the body and resting on or under the wing. As indicated by the increased thirst, the body temperature rises several degrees, to as high as 112° F., until just before death, when it may drop as low as 103° F.

Often birds die without having shown any previous clinical signs, but usually they linger for a day or two after the signs appear. Several outbreaks may occur in a flock in a single season, or the original one may be acute and last for only a few days. Intermittent outbreaks are more liable to occur if the birds are left on the originally infected premises or have constant contact with carrier chickens or turkeys. The initial outbreak usually causes the heaviest mortality, which is followed by intermittent recurrence in a few birds, with a low mortality at each subsequent flare-up of the disease. Although the average mortality in four outbreaks studied was 26.5 per cent, heavier losses have often been reported. One flock owner lost 169 out of 175 turkeys during the fall and winter in intermittent outbreaks. In very young poults the signs, course, and mortality are similar to pullorum disease.

Necropsy findings. The lesions resemble those observed in chickens. Because of the short duration of the disease, the birds nearly always die while in good flesh. The muscles of the breast have a tendency to be congested and often appear as if partially cooked. The heart is usually swollen and contains small, grayish necrotic areas or petechiae; in a few cases both have been observed. The liver is friable and is consistently enlarged to two or three times its normal size; it is bronze- to mahogany-colored or covered with a mixture of bronze- and mahogany-colored streaks. Pinpoint areas of necrosis have been noted. On cutting the organ, the blood flows readily. The spleen is always enlarged to two or three times its normal size, is friable, and appears mottled. In most birds the lungs present a parboiled appearance and often are more firm than normal because of minute caseated abscesses. The kidneys are usually enlarged and may show some petechiae.

The crop usually contains food, which suggests paralysis of the digestive tract, since birds seldom eat after clinical signs appear. The mucous membrane of the proventriculus sloughs readily. The gizzard contains food, and the lining is easily removed. With a few exceptions the intestine appears anemic when viewed from the exterior, and ulcerations of the mucous membrane may be visible through the serosa. Ulceration is most severe in the duodenum; a few ulcers from 1.0 to 4.0 mm. in diameter have been observed throughout the intestine, extending to the ceca.

The enlarged mahogany- or bronze-streaked liver, the enlarged spleen, the area of necrosis in the heart, and the grayish lungs are pathognomonic. Hemorrhagic enteritis, especially of the duodenum, and marked ulceration of the intestine, although uncommon in chickens, are more or less consistent lesions in turkeys. *Salmonella gallinarum*, the causative organism, can readily be isolated from all organs. In birds that have been dead for some time, pure cultures are more easily isolated from the bone marrow than from the liver, spleen, and heart blood.

In young poults Johnson and Pollard (1940) described the following necropsy findings: an increased percentage of large retained yolks, slightly enlarged somewhat friable liver of a white creamy color, with the surface mottled with slight hemorrhagic areas, and slight congestion in the anterior duodenum. The crops, gizzards, and intestines were always devoid of food, indicating lack of appetite for several hours before death. In adult carriers, there is, as in the case of pullorum disease, a predilection for the reproductive organs (Fig. 41.27).

Prevention, control, and treatment. Since chickens are apparently the most common carriers of the disease to turkeys, the two species should never be allowed to mingle. It is equally important to keep turkeys from yards or ranges that have recently been used for chickens.

Survivors of an outbreak should not be

FIG. 41.27 — Ovary from turkey hen, showing affected ova caused by fowl typhoid. No normal ova were present. (Hinshaw, Univ. of Calif.)
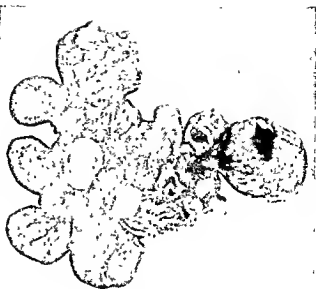
kept for breeders because of the danger of transmission of the disease through the egg.

It has been the general experience of research workers that drugs which are effective in reducing mortality are not effective in eliminating carriers.

Poults for replacements should be purchased only from hatcheries which can guarantee the poults to be free of the disease, i.e., U.S Pullorum-Typhoid Clean or Passed or of an equivalent grade (Nat. Poultry and Turkey Improvement Plans, 1963).

Control depends upon eliminating the infection in the flock. The removal of all sick birds and the transfer of the well birds to a new range that has not been used for either chickens or turkeys is recommended. One method for separating sick birds from well ones in an acute outbreak is to take the temperatures of all birds in the flock and eliminate those showing temperatures above 108° F.

The successful control of fowl typhoid in turkeys by the use of furazolidone, if given early in an outbreak, has been reported by Grumbles *et al.* (1954) and Cosgrove (1954). Grumbles *et al.* found that 100 gm. of the drug per ton of feed given as late as 3 days following exposure

prevented mortality. When given at the rate of 50 gm. per ton continuously beginning 3 days before exposure, at the time of exposure, and 3 days after exposure, mortality was prevented, but this amount did not provide a desirable degree of protection if started 5 days after exposure. Cosgrove stopped mortality in field outbreaks by giving feed containing 100 gm. per ton for 3 days and then feeding 50 gm. per ton until the disease was under control. This drug is relatively insoluble in water so must be given in the feed or administered individually. Grumbles reported that in a limited number of trials sick turkeys were successfully treated with 100-mg. capsules of furazolidone per bird *per os*. Attention is called to the observations of Hall and Cartrite (1961) and Stuart *et al.* (1963) that continuous feeding of furazolidone may encourage the development of resistant strains of *Salmonella gallinarum*.

In vitro and *in vivo* (with chickens) studies on the sensitivity of *S. gallinarum* to antibiotics (Glantz and Gordeuk, 1955) showed that chloramphenicol and aureomycin are of value against this disease in chickens. Chloramphenicol given at the rate of 200 mg. per bird per day *per os* or 1 to 2 gm. per pound of feed gave ex-

cellent protection if started on the day of infection. A relapse occurred when treatment was discontinued. Aureomycin at a level of 1 gm. per gallon of water was not effective. When it was given at the rate of 1 gm. per pound of feed, losses were only reduced to 25 per cent, but there was no relapse when the treatment was discontinued. Richey and Morgan (1959) successfully controlled artificially produced fowl typhoid in poults by administration of chloromycetin at the rate of 1.0 gm. per pound of feed providing treatment was started 5 days before inoculation and continued for 10 days. Treatment did not prevent development of carriers in survivors.

As the greatest source of the spread of the disease is the droppings, the roosts

should be screened to prevent the birds from having access to them. Sick birds should be taken out of the flock as soon as noted; frequent changes of the watering and feeding areas should be made; and whenever many new cases appear, the flock should be moved again to new quarters.

Next to the droppings, the greatest sources of infection are the food and water containers. They should be cleaned and disinfected daily or even oftener. In the absence of running water, fresh, clean water should be given several times daily.

Experimental work has not demonstrated that vaccination with fowl-typhoid bacterins is effective for preventing or controlling the disease in turkeys.

REFERENCES

Boney, W. A.: 1947. Isolation of *Shigella gallinarum* from turkey eggs. Am. Jour. Vet. Res 8:133.

Cosgrove, A. S.: 1954. Clinical investigation of furazolidone in the treatment of fowl typhoid Vet. Med, 49:393.

Glantz, P. J., and Cordeuk, Jr.: 1955. *In vitro* and *in vivo* sensivity of the fowl typhoid organism, *S. gallinarum*, to antibiotics. Poultry Sci. 34:880.

Grumbles, L. C., Wills, F. K., and Boney, W. A. Jr.: 1954. Furazolidone in the treatment of fowl typhoid in turkeys. Jour, Am. Vet. Med. Assn. 124:217.

Hall, C. F, and Cartrite, H. T.: 1961. Observations on strains of *Salmonella gallinarum* apparently resistant to furazolidone. Avian Dis. 5:382.

Hinshaw, W. J.: 1930. Fowl typhoid in turkeys. Vet. Med. 25:514.

———, and Taylor, T. J.: 1933. A chronic carrier of fowl typhoid of turkeys. Jour. Am. Vet. Med. Assn. 82:9222.

Johnson, E. P., and Pollard, M.: 1940. Fowl typhoid in turkey poults. Jour, Am. Vet. Med. Assn. 96:243.

Kaupp, B. F., and Dearstyne, R. S.: 1924. Fowl typhoid—a comparison of various European strains with those of North America. Poultry Sci. 3:119.

Martinaglia, G.: 1929. A note on *Salmonella gallinarum* infection of ten-day-old chicks and adult turkeys. Jour. South African Vet. Med. Assn. 1:35.

National Poultry and Turkey Plans, and auxiliary provisions: 1963. USDA, ARS Misc. Publ. 759.

Pfeifer, W., and Roepke, W.: 1917. Zweite Mitteilung über das Auftreten des Huhnertyphus und die Eigenschaften seines Erregers. Zentralbl. f. Bakt. I. Orig. 79:125. (Abst. in Jour Comp. Path. and Therap. 30 263.)

Richey, D. J., and Morgan, C. L.: 1959. The treatment of *Salmonella gallinarum* infection in turkey poults with chloramphenicol and furazolidone. Am. Jour. Vet. Res. 20:659.

Stuart, E. E., Keenum, R. D., and Bruins, H. W.: 1963. Experimental studies on an isolate of *Salmonella gallinarum* apparently resistant to furazolidone. Avian Dis. 7:294.

Vidovic, F.: 1931. Die Untersuchungen der Pathogenität des *Bacterium gallinarum* bei Huhnern und Puten. Inaug-Diss. Vet. Fakult. Zagreb. 1930. Zentralbl. f. Bakt. Ref. 105:472.

NEWCASTLE DISEASE

The reader is referred to the more complete discussion of this disease in Chapter 22. Turkeys are susceptible to Newcastle disease; the signs and pathology are similar to those seen in chickens.

Turkeys should not be reared in close proximity to chicken-rearing communities

where the disease is prevalent. If it is necessary to so rear them, vaccination of the turkeys should be considered.

SALMONELLOSIS (Paratyphoid)

This group of diseases is one of the major causes of losses, especially in young turkeys. This section deals with the types

other than *S. pullorum* and *S. gallinarum* infections (pullorum disease and fowl typhoid). For further reading, Chapter 9 and a review on salmonellosis by Edwards (1958b) are recommended. A revised, simplified scheme for serological typing has been compiled by Kauffmann and Edwards (1957), Kauffmann (1959), and Edwards (1962). Buxton (1957) gives a review of salmonellosis in animals. Especially valuable are the groupings of serotypes by isolations according to animal species contained in this reference.

Over 800 serotypes of Salmonellae have been described (Edwards and Ewing, 1962). The following list records 102 types which have been reported from turkeys. When the list was first compiled for the third edition in 1952, 62 types were recorded. From 1952 to 1959 an additional 19 types were added. Since the publication of the fourth edition in 1959, six more have been reported; these are *S. denver*, *S. java*, *S. johannesburg*, *S. livingston*, *S. manila*, and *S. uno*, all from United States (Moran, 1961).

No additional serotypes from other countries were found in the literature reviewed. Gordon and Tucker (1957) reported the isolation of *S. infantis* from a turkey poult in England and stated that this appeared to be the first isolation of this type from turkeys. It probably is the first published case, although *S. infantis* was listed from turkeys in the third edition. The report for that listing was obtained from Dr. P. R. Edwards in a personal communication. *S. infantis* is now frequently isolated from both turkeys and chickens in the United States. Moran (1961) lists 78 isolations from turkeys and 94 isolations from chickens for the period of January, 1957, to July, 1961. Dixon (1962) reported the isolation of *S. menston* from two turkeys in a survey made of two turkey processing plants in England. It was first reported by Colbeck *et al.* (1951) from England.

This list, probably incomplete, was compiled through the cooperation of research workers and diagnostic laboratory direc-

tors, and by a survey of the available worldwide literature. Earlier references include Edwards (1939), Saxer (1932), Nakamura *et al* (1939), Bruner and Moran (1949), Edwards and Hermann (1949), Bruner (1951, 1956), Edwards and McWhorter (1953), Edwards *et al.* (1954), Lukas and Bradford (1954), McWhorter and Edwards (1956), and Burr *et al.* 1957). Personal communications contributing to the list are acknowledged from Browne (1958), Bruner (1958), Dickinson (1958), Edwards (1958), Pomeroy (1958), Van Ryzin (1958), and Worcester (1958). Summaries of isolations of Salmonella serotypes from turkeys in England are given by Colbeck *et al.* (1951), Smith and Buxton (1951), and Buxton (1948, 1957). The long list of Salmonella types now known to be capable of producing disease in turkeys becomes more significant when one considers that the first report of losses in turkeys in the United States was made as recently as 1933 (Rettger *et al.*, 1933). In a summary by Edwards *et al.* (1948) over 2,200 additional outbreaks and 40 new types were added to the original list given by Edwards (1939). Of 19 types found in California outbreaks (Hinshaw *et al.*, 1944), *S. typhi-murium* accounted for 60 per cent. This type is still one of the most important causes of turkey losses. It is also one of the most common types isolated from other animals, including man. Saxer (1932) reported an outbreak caused by *S. enteritidis* (Gaertner type) after feeding turkeys meat from a calf suffering from navel infection. Nakamura *et al.* (1939) also reported an outbreak in Japan caused by *S. enteritidis*.

The ten most frequently reported types in turkeys in the United States from 1957 to 1961 according to Moran (1961) were *S. typhi-murium*, *S. san diego*, *S. anatum*, *S. newport*, *S. heidelberg*, *S. saint-paul*, *S. chester*, *S. munenchen*, *S. bredeney*, and *S. enteritidis*, in the order given.

Transmission. Evidence that this group of diseases may be transmitted through the egg in a manner similar to pullorum disease has been presented by Cherrington *et al.* (1937), who succeeded in iso-

lating *S. typhi-murium* from 2 of 6 ovaries removed from reacting turkeys, and from 3 of 30 "dead-in-shell" embryos. Lee *et al.* (1936) in an earlier paper reported the isolation of this organism from 4 of 10 ovaries removed from artificially infected turkey hens. *S. typhi-murium* has been isolated from both the ovary and oviduct of turkey hens by Hinshaw and McNeil (1943).

The incidence of *S. typhi-murium* in eggs laid by carriers is not high according to the literature available and our own experience. Pomeroy and Fenstermacher (1939) reported the isolation of paratyphoid organisms from 7 out of 200 incubated eggs that failed to hatch. *S. typhi-murium* was isolated from the ovaries of 2 of the 9 reactors that laid the above-mentioned eggs. Pomeroy and Fenstermacher (1941) showed that *S. typhi-murium* will pass through the unbroken eggshell and infect developing embryos, some of which hatch and become a source

of infection to normal poults. Bigland and Paps (1953), Frank and Wright (1956), and Wright and Frank (1956) have confirmed these results. Frank and Wright found that dipping eggs, artificially infected with *S. typhi-murium*, in sodium hydroxide at concentrations up to 2 per cent for 5 minutes failed to prevent penetration of the eggshells. They also showed that lower specific gravities (<1.070) favored penetration.

Hinshaw and McNeil (1943) reported that 81 per cent of adult *S. typhi-murium* carriers yielded the organism from the intestines, in contrast to 17 per cent which yielded it from the reproductive organs. Gauger and Greaves (1946) made similar observations in attempts to isolate *S. typhi-murium* from eggs laid by naturally and artificially infected hens. In their studies a much higher percentage of positive isolations was made from the outside of the shells than from egg contents. Likewise, necropsy of the birds showed a

LIST OF SALMONELLA ISOLATED FROM TURKEYS

S. alachua	S. derby	S. madelia	S. saint-paul
S. albany	S. dublin	S. manhattan	S. san-diego
S. amager	S. duesseldorf	S. manila	S. schwarzengrund
S. amherstiana	S. eastbourne	S. meleagridis	S. siegburg
S. anatum	S. enteritidis	S. menston	S. senftenberg
S. banana	S. edinburg	S. mgulani	S. simsbury
S. bareilly	S. fresno	S. minneapolis	S. stanley
S. berkeley	S. florida	S. minnesota	S. takoradi
S. berta	S. gaminara	S. mission	S. taksony
S. binza	S. give	S. montevideo	S. tel-aviv
S. blockley	S. grumpensis	S. muenchen (oregon)	S. tennessee
S. bovis-morbificans	S. hamilton	S. muenster	S. thomasville
S. brancaster	S. harrisonburg	S. new-brunswick	S. thompson
S. braenderup	S. heidelberg	S. newington	S. typhi-murium
S. bredeney	S. illinois	S. newport (puerto)	S. typhi-murium
S. budapest	S. indiana	S. onderstepoort	var. copenhagen
S. california	S. infantis	S. oranienburg	S. uganda
S. cambridge	S. irumu	S. orion	S. uno
S. canoga	S. java	S. panama (italiana)	S. urbana
S. cerro	S. javiana	S. paratyphi-B	S. vejle
S. chester	S. johannesburg	(schottmuelleri)	S. westhampton
S. cholera-suis	S. kaapstad	S. pomona	S. wichita
S. concord	S. kentucky	S. poona	S. worcester
S. corvallis	S. kingston	S. reading	S. worthington
S. cubana	S. lexington	S. rutgers	
S. denver	S. litchfield	S. rubislaw	
	S. livingston		
	S. london		

higher incidence of *S. typhi-murium* isolations from the digestive tract than from the reproductive tract.

From the information available it would seem that the important means of egg contamination and subsequent infection of the poult is from infected intestinal contents coming in contact with the shell during expulsion from the body or in the nest. Ovarian transmission, however, must not be ignored.

Boyer *et al.* (1962) discuss the role of Salmonella-contaminated feed in the transmission of the disease. They conclude that this is an important source of the disease in turkeys and stress the need for eliminating this source of infection.

Yamamoto *et al.* (1961) made an experimental study of *S. typhi-murium* infection in market-age turkeys. The turkeys used in the experiment were infected by inoculations into the crop, and were kept under observation for 33 to 44 days. At the end of 14 days 83.4 per cent were shedding the organism in feces, and 27.8 per cent were still shedders at the termination of the experiment. At necropsy, one of 18 birds yielded a positive culture from the reproductive tract in contrast to 11 positive cultures isolated from the intestinal tract. Shells of eggs laid yielded positive cultures but contents of incubated eggs were negative. There was a 38.8 per cent correlation of serologic response and positive culture isolations at the termination of the trial.

Signs. The signs in young poults are indistinguishable from pullorum disease. The age at which poults may be affected ranges from a few days after hatching to maturity. In general, however, the age incidence is that of pullorum disease—from 5 or 4 days of age to 1 month. The age at which the disease is first observed in poults will depend on whether the poults are infected while in the incubator or after being placed in the brooder. In one outbreak studied, symptoms were seen 2 days after the poults were taken from the incubator, indicating transmission in the incubator soon after the eggs had started to hatch.

Diarrhea in young poults is not constant and often poults normal in the evening may be found dead in the morning. Where death is delayed for several days, weakness, unthriftiness, sagging wings, and diarrhea are characteristic symptoms. Many poults that survive for several days will become emaciated, and the feathers around the vent will be matted with fecal material. Higgins *et al.* (1944) reported inflammation and swelling of the leg joints of poults suffering from *S. enteritidis* infection.

In older turkeys, loss of appetite, unthriftiness, loss of flesh, and a general unkempt appearance have been most commonly observed. Diarrhea may or may not be in evidence. Death usually follows after several days of sickness.

Necropsy findings. Inflammation of the duodenum, congestion of the liver, kidney, gallbladder, and heart muscle are the most constant postmortem findings. The pericardial sac is often filled with a serous straw-colored fluid. Another common finding is a ceeal plug similar to that sometimes seen in pullorum disease. Lung and heart lesions are rare, but air-sac involvement is common.

In adult turkeys, marked inflammation of the intestine with occasional necrotic ulcers is seen. The liver and spleen in these cases are usually swollen and congested. Diagnosis depends on isolating and identifying the causal organism.

Prevention. Prevention consists in obtaining stock which is free of the disease and in preventing the birds from being exposed to other animal reservoirs of infection. Salmonella-contaminated feed must also be avoided. Most of the types of Salmonella responsible for losses in turkeys are also prevalent in other animals including man. Thus the program of prevention must be extended to all animals on the ranch. Eradication of rats, mice, flies, and reptiles is essential. If the disease is diagnosed in any group of poults, these poults should be reared separately from other groups. Such infected groups should never

be used for breeders.

The use of the agglutination test as an aid in determining the presence or absence of the disease on the premises will depend on the facilities available for having such tests made. Each ranch must be handled as an individual unit when making plans for testing. A separate test must be made for each species isolated, and complete knowledge of the disease history of the flock is essential to a successful program. The agglutination test for the paratyphoids is more complicated and, as now conducted, more subject to variation than is the one for pullorum disease. To conduct it properly, the laboratorian must be thoroughly familiar with the antigenic structure of Salmonellae and be able accurately to interpret results. It is absolutely necessary to know the type causing the disease, and, as may often be the case, there may be two or more species responsible (Edwards and Bruner, 1940). If the complete history is known and a competent laboratory is available, testing may be advised (Hinshaw and McNeil, 1943; McNeil and Hinshaw, 1951; Yamamoto et al., 1962).

A Salmonella and Arizona typing center for strains isolated from nonprimate animal sources has been established by the United States Department of Agriculture at Ames, Iowa. The mailing address for the Center is Salmonella Typing Center, U.S.D.A.-A.R.S., National Animal Disease Laboratory, Box 70, Ames, Iowa 50011.

According to Williams (Chapter 9), at least three states, Minnesota, California, and Texas, have official rules and regulations including a blood-testing program for control of S. typhi-murium infection. The California system is described by Delay et al. (1955) and Goetz (1962). In any program, the agglutination test should be only used to locate diseased flocks and these should be eliminated from the breeding program.

Preincubation fumigation of hatching eggs is being used by many breeder flock owners as a preventive measure to reduce the chances of egg contamination. The method described by Stover (1960) is in general use for this procedure. For details see Chapters 5 and 9.

Hatcheries and egg-selling groups can help prevent the group of diseases from spreading by keeping thoroughly familiar with all the ranches furnishing hatching eggs. Frequent use of the diagnostic laboratory is urged during the brooding season in order to insure a high percentage of diagnoses of the outbreaks which occur. Whenever a diagnosis is made, the owner should be made familiar with the problem and responsibility he has in preventing the spread of the disease. Often, when only one brood is affected, it will be good insurance to destroy all the survivors. In any case, survivors of an outbreak normally should not be used for breeding purposes.

Other animal and bird reservoirs on the ranch must also be eliminated if the disease is to be eradicated. Cats, flies, and even snakes and lizards are important carriers of this group of diseases, and should not be overlooked in outlining the control program (McNeil and Hinshaw 1944; Hinshaw and McNeil, 1945, 1947).

Treatment and control. Pomeroy et al. (1948) reported that sulfathiazole, sulfaguanidine, and sulfadiazine are about one-half as effective in reducing losses from S. typhi-murium infection among poults as compared with chicks similarly infected and treated. In general the sulfonamides are more toxic for poults than for chicks and must, therefore, be used with caution.

Furazolidone was used successfully by Smith (1955), Wilson (1955), and Bierer and Vickers (1960) for treatment of experimentally produced S. typhi-murium infection in turkey poults and chicks. The usual practice in the United States, according to Sieburth (1957a), is to use a 0.005 per cent level continuously for prevention and a 0.01 per cent level for treatment of Salmonella infections. Sieburth also reported that furazolidone at these levels suppressed the formation of

agglutinins but not the formation of indirect hemagglutinins (Sieburth, 1957b) to *S. typhi-murium* in orally infected chickens. Because of his findings, the use of this drug should be avoided until completion of pullorum and paratyphoid testing programs.

These drugs have not proven of value in eliminating carriers from an infected flock, and should only be used for the purpose of salvaging as many as possible survivors. Salvaged birds should not be sold for meat purposes until thoroughly recovered from the disease.

West *et al.* (1945) studied the effect of streptomycin *in vitro* on 412 cultures of all the recognized types of Salmonella. The majority of the strains required from two to four times as much streptomycin to inhibit growth as did a standard test strain of *E. coli*. Bacterins for prevention and control cannot be recommended. The multiplicity of types makes the general use of bacterins as impossible as a generalized testing program.

See Chapter 9 for a detailed discussion on control and treatment.

REFERENCES

Bierer, B. W., and Vickers, C. L.: 1960 Nitrofuran medication for experimental *Salmonella typhimurium* infection in poults. Avian Dis 4:22.

Bigland, C. H., and Papas, G 1953. Experiment in egg penetration by Salmonella. Canad. Jour. Comp. Med. 17:105

Boyer, C. I., Jr., Narotsky, S., Bruner, D. W., and Brown, J. A.: 1962 Salmonellosis in turkeys and chickens associated with contaminated feed. Avian Dis. 6:43.

Browne A S 1958 Calif St Health Dept., personal communication.

Bruner, D. W.: 1931. *Salmonella kingston*: a new type. Cornell Vet. 41:339.

——— 1956 *Salmonella* presented for identification during the 5-year period 1950-1954. Cornell Vet. 46 11

——— 1958 Cornell University, personal communication.

——— and Moran, A. B 1949. *Salmonella canoga*—a new type. Jour. Bact. 57:135.

Burt, W E., Tourtellotte, M. Lugenbuhl, R. E., and Junghere, E. L.: 1957. Salmonella heidelberg infection as a problem in pullorum disease control. Avian Dis. 1:298.

Buxton, A: 1918. Avian salmonellosis in England and Wales. Proc. Eighth World's Poultry Cong Copenhagen, Aug. 20-27 1:609.

——— 1957 Salmonellosis in animals Review Series No. 5, Commonwealth Bur. Anim. Health, Commonwealth Agr Bureaus, Farnham Royal, Bucks, England.

Cherrington, V A., Gildow, E M., and Moore, P.: 1937. Paratyphoid in turkeys. Poultry Sci. 16 226

Colleck, J C., Douglas, S H., and Taylor, J 1958. A new *Salmonella* type. *Salmonella mension.* Jour Path. and Bact (Brit) 63 751

Delay, P. D., Jackson, T. W., Stover, D. E., Jones, E. E., and Worcester, M. W.: 1955. A testing service for the control of *Salmonella typhimurium* infection in turkeys—A progress report. Jour Am Vet Med. Assn 127 435.

Dickinson, E M: 1958 Oreg St Coll., personal communication.

Dixon, J M S : 1962. Salmonellae in two turkey processing plants. Monthly Bul. Minist. Health and Pub Health Lab. Service (Brit) 21:158.

Edwards, P R : 1959 Incidence of Salmonella types in fowls in the United States. Proc. Seventh World's Poultry Cong., p 271

——— 1956. *Salmonella* and salmonellosis Ann N.Y. Acad. Sci 66:44.

——— 1958a U S Public Health Service, personal communication.

——— 1958b. Salmonellosis Observations on incidence and control. Ann. N.Y. Acad. Sci. 70 598.

——— 1962 Serologic examination of Salmonella cultures for epidemiologic purposes. USPHS, CDC, Atlanta, Georgia August 1962, p. 1

——— Browne, A S., McWhorter, A C., and Williams, A.: 1954. A new Salmonella serotype (9, 12,238) isolated from turkeys Cornell Vet 44:259. (Note: this type now called *S. fresno*)

——— and Bruner, D W 1943 The occurrence of multiple types of paratyphoid bacilli in infections of fowls, with special reference to two new Salmonella species. Jour. Infect. Dis. 66,218.

——— Bruner, D. W., and Moran, A. B.: 1948 The genus Salmonella: its occurrence and distribution in the United States. Ky Agr Exper Sta., Bul. 525

——— and Ewing, W. H.: 1962 Identification of Enterobacteriaceae. Burgess Publ. Co., Minneapolis.

——— and Hermann, G. J 1949 Two new Salmonella types: *Salmonella corvallis* and *S. colorado.* Jour. Bact. 58 111

————, and McWhorter, A. C.: 1953. Two new Salmonella types: S. harrisonburg and S. westhampton. Cornell Vet. 43:110.

Frank, J. F., and Wright, G. W.: 1956. The disinfection of eggs contaminated with Salmonella typhimurium. Canad. Jour. Comp. Med. 20:406.

Gauger, H. C., and Greaves, R. E.: 1946. Bacteriological examination of shells and contents of eggs laid by turkeys naturally or artificially infected with S. typhimurium. Poultry Sci. 25:119.

Goetz, M. E.: 1962. The control of paracolon and paratyphoid infections in turkey poults. Avian Dis. 6:93.

Gordon, R. F., and Tucker, J. F.: 1957. The isolation of Salmonella infantis from a turkey poult. Monthly Bul. Minist. Health and Pub. Health Lab. Service (Brit.). 16:71.

Higgins, W. A., Christiansen, J. B., and Schroeder, C. H.: 1944. A Salmonella enteritidis infection associated with leg deformity in turkeys. Poultry Sci. 23:340.

Hinshaw, W. R., and McNeil, E.: 1943. The use of the agglutination test in detecting Salmonella typhimurium carriers in turkey flocks. Proc. 47th Ann. Meet. U.S. Livestock Sanit. Assn., p. 106.

————, and McNeil, E.: 1945. Salmonella types isolated from snakes. Am. Jour. Vet. Res. 6:264

————, and McNeil, E.: 1947. Lizards as carriers of Salmonella and paracolon bacteria, Jour. Bact. 53:715.

————, McNeil, E., and Taylor, T. J.: 1944. Avian salmonellosis. Types of Salmonella isolated and their relation to public health. Am. Jour. Hyg. 40:264.

Kauffmann, F.: 1959. Supplement to the simplified Kauffmann-White schema. Acta Pathologica et Microbiol. Scandinavica 45:406.

————, and Edwards, P. R.: 1957. A revised, simplified Kauffmann-White schema. Acta Pathologica et Microbiol. Scandinavica 41:242.

Lee, C. D., Holm, G., and Murray, C.: 1936. Paratyphoid infection in turkeys. Jour. Am. Vet. Med. Assn. 89:65.

Lukas, G. N., and Bradford, D. R.: 1954. Salmonellosis in turkey poults as observed in routine necropsy of 1,143 cases. Jour. Am. Vet. Med. Assn. 125:215.

McNeil, E., and Hinshaw, W. R.: 1944. Snakes, cats, and flies as carriers of Salmonella typhimurium. Poultry Sci. 23:456.

————, and Hinshaw, W. R.: 1951. Procedures for conducting the agglutination test for detection of Salmonella carriers in turkey flocks. Vet. Med. 46 360.

McWhorter, Alma G., and Edwards, P. R.: 1956 A new Salmonella serotype (Salmonella rutgers). Cornell Vet. 45:509.

Moran, A. B.: 1961. Occurrence and distribution of Salmonella in animals in the United States. Proc. 65th Ann. Meet. U.S. Livestock Sanit. Assn., p. 441.

Nakamura, N., Nose, Y., and Negishi, B.: 1939. An outbreak of Salmonella enteritidis infection in baby turkey poults. Proc. Seventh World's Poultry Cong., p. 240.

Pomeroy, B. S.: 1958. University of Minnesota, personal communication.

————, and Fenstermacher, R.: 1959. Paratyphoid infection of turkeys. Jour. Am. Vet. Med. Assn. 94:90.

————, and Fenstermacher, R.: 1941. Paratyphoid infection of turkeys. Am. Jour. Vet. Res 2:285.

————, Fenstermacher, R., and Roepke, M. H.: 1948. Sulfonamides in the control of salmonellosis of chicks and poults. Jour. Am. Vet. Med. Assn. 112,296.

Rettger, L. F., Plastridge, W. N., and Cameron, R.: 1933. Endemic paratyphoid infection in turkeys. Jour. Infect. Dis. 53:272.

Saxer, E.: 1932. Gärtnerinfektion bei Truthühnern. Schweizer Archiv für Tierheilk. 74:351. (Abst. from Internat. Rev. Poultry Sci. Vol. 5 (3/4) 96. 1933.)

Siebarth, J. M.: 1957a. The effect of furazolidone on the cultural and serological response of Salmonella typhi-murium infected chickens. Avian Dis. 1:160.

————: 1957b. Indirect hemagglutination studies on salmonellosis of chickens. Jour. Immunol. 78:580.

Smith, H. W.: 1955. The treatment of experimental Salmonella typhimurium infection in turkey poults and chicks. Vet. Record 67:749.

————, and Buxton, A.: 1951. An outbreak of Salmonella schwarzengrund infection in poultry. Jour. Path. and Bact. (Brit.) 63:459.

Stover, D. E.: 1960. Fumigation of hatching eggs. Calif. State Dept. of Agr. Bul. 49.30.

Van Krijnin, R. J.: 1958. Mich. St. Univ. personal communication.

West, M. G., Doll, E. R., and Edwards, P. R.: 1945. Inhibition of Salmonella cultures by streptomycin. Proc. Soc. Exper. Biol. and Med. 60.363.

Wilson, J. E.: 1955. The use of furazolidone in the treatment of infections of day-old chicks with S. pullorum, S. gallinarum, S. typhimurium, and S. thompson. Vet. Record 67:849

Worcester, W. W.: 1958. Calif. St. Dept. Agr., Sacramento, Calif., personal communication.

Wright, G. W., and Frank, J. F.: 1956. Penetration of eggs by Salmonella typhi-murium. Canad Jour. Comp. Med. 20:453.

Yamamoto, R., Adler, H. E., Sadler, W. W., and Stewart, G. F.: 1961. A study of Salmonella typhimurium infection in market-age turkeys. Am. Jour. Vet. Res. 22:382.

————, Killian, J. G., Babcock, W. E., and Dickinson, E. M.: 1962. Some observations on serological testing for Salmonella typhimurium in breeder turkeys. Avian Dis. 6:444.

Arizona Infections (Paracolon Infections)

For a more complete discussion on paracolon infection of birds, the reader is referred to Chapter 9. The diseases included in this group are caused by Gram-negative bacteria which are serologically and biochemically related. They occupy a position between the coliforms and Salmonellae and possess characteristics common to both.

Paracolon types belonging to the Arizona group (Edwards et al., 1947, and Edwards et al., 1956) are most often the cause of disease in turkeys They cause

clinical signs indistinguishable from those seen in salmonellosis. Eye involvement is common in this disease. Reports on paracolon infections have been made by a number of research workers including Hinshaw and McNeil (1944, 1946), Bruner and Peckham (1952), Goetz and Quortrup (1953), and Goetz et al. (1954, 1955).

Prevention, control, and treatment are the same as for salmonellosis. Transmission through the egg has been proven, so it is essential that infected flocks are not kept for breeders.

REFERENCES

Bruner, D. W., and Peckham. M. C. 1952. An outbreak of paracolon infection in turkey poults. Cornell Vet. 42 22.

Edwards, P. R., McWhorter, A. C., and File, M. A. 1956. The Arizona group of Enterobacteriaceae in animals and man Bul World Health Organization. 14 511.

——, West, M. G., and Bruner, D. W. 1947. Arizona group of paracolon bacteria. A new group of bacteria pathogenic for animals and poultry for man. Ky. Agr. Exper. Sta., Bul. 499 1.

Goetz, M. E., Dunsing, J. W., and Quortrup, E. R. 1955. Paracolon infections of turkeys in San Diego County. Calif. Vet. 8 (May–June), p. 31.

——, and Quortrup, E. R. 1953 Some observations on the problems of Arizona paracolon infections of poults. Vet. Med 48 59

——, Quortrup, E. R., and Dunsing, J. W. 1954. Investigations of Arizona paracolon infections in poults Jour. Am. Vet. Med. Assn. 124:120.

Hinshaw, W. R., and McNeil, E. 1944. Gopher snakes as carriers of salmonellosis and paracolon infections. Cornell Vet 34:248.

——, and McNeil, E. 1946 The occurrence of type 10 paracolon in turkeys. Jour. Bact. 51:281.

PULLORUM DISEASE*

This disease, caused by Salmonella pullorum, has been increasing in economic importance to the turkey industry since the advent of the commercial hatching of turkey eggs. It was first described in turkeys by Hewitt (1928) in Minnesota and has since become widespread among turkeys in America as well as in some of the other countries of the world. Comprehensive reviews of the literature have been given by Tittsler (1932), Johnson and Anderson (1936), Hinshaw (1939), and Carpenter et al. (1949). Investigators in other countries who have reported on the disease in turkeys include Dalling et al. (1929), Jansen (1932), and Barboni (1937).

Previous to 1938 when Johnson and Anderson reported an outbreak which ap-

parently originated from eggs laid by turkey carriers, all the evidence pointed to chickens as the main source of the infection in turkeys. The disease, however, soon became established in many turkey flocks, and the cycle of infection has been shown to be identical to that for chickens (Hinshaw et al., 1942; Carpenter et al., 1949; and Gwatkin and Dzenis, 1953).

Signs. The clinical signs in poults are similar to those described for chicks. The disease is usually acute, and many poults die without showing noticeable signs. Sick poults seem cold and sit around the hot part of the hover space. Their wings sag, their heads hang, and their feathers appear unkempt. The skin over the feet and legs usually appears dry and somewhat wrinkled. Diarrhea may or may not be present; but in most of the cases that are prolonged for 2 or 3 days, diarrhea is in-

* See also Chapter 8.

dicated by the pasting of the down around the vent. Labored breathing, due to pneumonia, is commonly observed.

Course and mortality. Most of the losses occur during the first 3 weeks after hatching and may start as early as the second day. It is not uncommon for relapses to occur at any time up to maturity, with varying degrees of mortality. Frequently, when survivors of an early age outbreak reach 9 to 10 weeks of age and are transferred to a growing ration or moved to new quarters, a second outbreak occurs with a subsequent mortality of 5 to 15 per cent. Outbreaks may occur in turkeys 3 to 6 months of age. Losses of this age group have, as a rule, been small. Subacute outbreaks have been experienced in breeding flocks after they are in production. Such outbreaks are attributed to transmission by eating infected eggs and subsequent spread by the intestinal shedders of the organisms.

The mortality in poults under 1 month of age varies from less than 10 to as high as 100 per cent of a brood, depending on the virulence of the organism and the management of the brood.

Necropsy findings. Minute caseous abscesses in the lungs and heart muscles similar to those seen in chicks are the most characteristic lesions. Similar abscesses may be found in the gizzard muscles. The intestines usually lack tone and contain an excessive mucous discharge. Cecal cores are seen, but they are not pathognomonic. The liver is often congested and swollen and may be of an ocher to a bronze color streaked with areas of congestion. Pin-point areas of necrosis are common.

The postmortem findings in partially grown poults are similar to those seen in younger ones, but these are usually less pronounced. Lung lesions are only occasionally seen, but necrotic foci in the liver are frequent findings, as are nodules in the gizzard and catarrhal enteritis.

The lesions seen in adult carriers are similar to those seen in carrier chickens and are principally confined to the re-

productive tract. S. pullorum has also been isolated occasionally from the lungs, intestines, bursa of Fabricius, and liver, and in one instance from the testes of reactors. A frequent finding in adults that have been killed in subacute outbreaks is marked ascites. In these cases as much as 1,000 cc. of fluid containing yellowish caseated flaky masses may be removed from a single bird. As a rule, such specimens yield S. pullorum from all tissues including the intestines.

Prevention. The first prerequisite in a program of prevention is to establish a source of pullorum-disease-free eggs. The second is to have such eggs hatched in a hatchery that accepts eggs only from pullorum-disease-free flocks of turkeys, chickens, and other fowl. The third is to brood and rear the poults in brooders with equipment that has had no contact with birds of any species that have had pullorum disease. If these three prerequisites are followed together with a good management program, there is little danger that pullorum disease will become established in a flock of turkeys.

When an outbreak occurs, it is recommended that the survivors be marked and reared separately from broods that have not had the disease. Such groups of survivors should be sold for market and never kept for breeders. They should be marketed before they start to lay eggs. The remainder of the birds on the premises, if they are to be kept for breeders, should be tested by means of the tube agglutination test. According to Hinshaw *et al.* (1942), this test, using a 1:25 dilution, made according to the procedure recommended in the National Poultry and Turkey Improvement Plans and Auxiliary Provisions (1963) is a reliable aid in locating pullorum-disease-free flocks. Hinshaw *et al.* (1910) reported that the whole-blood stained antigen test was 50 per cent as efficient as the standard tube test for detecting carriers of S. pullorum. Several investigators such as Bushnell (1915), Corpron *et al.* (1917), Gauger (1917), Garland *et al.* (1919), and Wright *et al.* (1957)

FIG. 41.28 — (A) Type of catching chute for handling large numbers of turkeys for vaccination, blood collection, or other purposes. (B) The same arrangement showing how a bird can be removed from the side, by reaching through the burlap "fence," without disturbing the other turkeys. (Hinshaw, Univ. of Calif.)
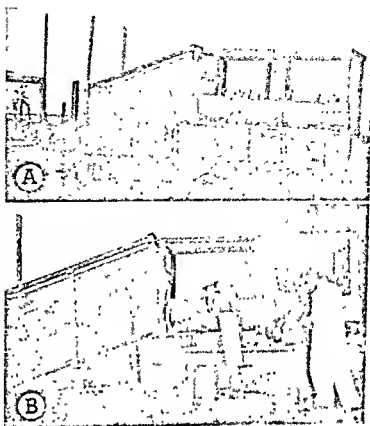
FIG. 41.29 — Pullorum disease. Method of withdrawing a blood sample from the median vein of the wing. (Hinshaw, Univ. of Calif.)

These rinse waters should be boiled before use and should be changed frequently.

An efficient crew consists of the operator and at least two assistants: one for banding the birds and one for recording the band numbers, emptying the syringes, and cleaning them. If speed is to be maintained, the operator should only have to withdraw the blood. It is, therefore, necessary that the flockowner furnish ample help in order always to have a bird on the table ready to be bled. Figures 41.30, 41.31, and 41.32 illustrate a few of the steps in this technique.

Control and treatment. There is no practical method of control or treatment once the disease has become established in a brood. Daily cleaning and the removal of all sick and dead poults several hours daily will aid in preventing its spread

FIG 41.30 — Pullorum disease Equipment used by a bleeding crew for collecting blood samples by the syringe technique. (Hinshaw, Univ. of Calif.)

Increasing the heat in the brooder may be helpful in preventing excessive loss. Cleaning and disinfecting the water fountains and feed hoppers several times daily and the use of fresh, unadulterated water are also recommended.

Bottorff and Kiser (1947), Mullen (1946), Anderson (1946), and Pomeroy et al (1948) have shown that mortality can be reduced by the use of sulfonamides

FIG. 41.31 — Type of rack used by the California Poultry Improvement Advisory Board for holding blood samples during the bleeding procedure. (Hinshaw, Univ. of Calif.)

given at the rate of 0.25 to 2.0 per cent in the mash for periods up to a week. Drugs tried by these investigators include sullamerazine, sulfadiazine, sulfamethazine, sulfapyrazine, and sulfaguanidine. Complete prevention of losses has not been reported. Pomeroy et al. concluded that the sulfonamides tried by them were of little or no value in reducing mortality among poults experimentally infected with S. pullorum.

For reviews on the value of antibiotics and other recently introduced drugs for treatment of Salmonella infection the reader is referred to the sections on salmonellosis, fowl typhoid, and Chapter 8.

Treatment with drugs should be made only after a positive diagnosis has been made, and then only upon the advice of a competent veterinarian. *Carriers are not eliminated by drugs, so their use is not a substitute for a testing program.*

Every precaution should be taken to prevent contact of an infected brood with other broods that are to be brought into the house after the outbreak is in progress. The brood suffering from the disease should be kept in isolated quarters. Under no circumstances should equipment used for the infected brood be used

FIG. 41.32 — Pullorum disease. A crew at work bleeding a flock of turkeys. (Hinshaw, Univ. of Calif.)

for later hatches until it has been thoroughly cleaned and disinfected.

When the disease has run its course, the survivors should be toe-marked and raised separately from the other lots. *None of the survivors should be saved for breeding purposes.* The survivors should be marketed as soon as they are in condition and the breeders selected from groups that have not suffered from the disease. These breeders should be tested as described under prevention.

REFERENCES

Anderson, G. W.: 1946. Sulfamerazine in the treatment of pullorum disease. Jour. Am. Vet. Med. Assn. 108:427.

Barboni, E.: 1937. Ricerche sul primo focolaio di pullorosi nei tacchini riscontrato in Italia. La Clin. Vet. 60.597.

Botteoff, C. A., and Kiser, J. S.: 1947. The use of sulfonamides in the control of pullorum disease. Poultry Sci. 26.535.

Bushnell, L. D.: 1945. Pullorum testing of turkeys. Poultry Sci. 24:508.

Carpenter, J. A., Anderson, G. W., Johnston, R. A., and Garrard, E. H.: 1949. Pullorum disease in turkeys. Poultry Sci. 28:270.

Corpron, R., Bivins, J. A., and Stafseth, H. J.: 1947. Pullorum disease studies in turkeys. Poultry Sci. 20:310.

Dalling, T., Mason, J. H., and Gordon, W. S.: 1929. Bacillary white diarrhea (B.W.D.): B. pullorum isolated from a turkey poult in England. Vet. Record 9.902.

Gatland, F. W., Jr., Winter, A. R., and Amlet, E. R.: 1949. A comparison of methods of testing turkeys for *Salmonella pullorum* infection. Poultry Sci. 28 63.

Gauger, H. C.: 1947. Comparison of the rapid whole-blood K-antigen and the tube agglutination test for the detection of pullorum disease in turkeys. Poultry Sci. 26 229.

Gwatkin, R., and Dzenis, L.: 1953. Studies in pullorum disease. 32. Infection of adult turkeys with single and multiple oral doses of mixed forms of *Salmonella pullorum*. Canad. Jour Comp. Med. 17:251.

Hewitt, E. A.: 1928. Bacillary white diarrhea in baby turkeys. Cornell Vet. 18.272.

Hinshaw, W. R.: 1939. Diseases of turkeys in United States—a review. Proc. Seventh World's Poultry Cong., p. 236.

——, Harr, J. F., and Niemezer, W. E.: 1910. Comparison of the tube and the Jones, E. E., whole blood tests for pullorum disease of turkeys. Cornell Vet. 30 50.

——, McNeil, E., and Taylor, T. J.: 1942. Four years' progress in eradication of pullorum disease from turkey flocks. Proc. 46th Ann. Meet. U.S. Livestock Sanit. Assn., p. 224.

Jansen, J.: 1932. Chronische pullorum-infectie bij volwassen kalkoenen. Tijdschr. voor Diergeneesk. 59:1047.

Johnson, E. P., and Anderson, G. W.: 1936. Pullorum disease in turkeys. Jour. Infect. Dis. 58:337.

Mullen, E. J.: 1946. Sulfamerazine as a prophylactic in pullorum disease. Jour. Am. Vet. Med. Assn. 108:163.

National Poultry and Turkey Improvement Plans and Auxiliary Provisions: 1963. U.S.D.A. Misc. Publ. No. 739.

Pomeroy, B. S., Fenstermacher, R., and Roepke, M. H.: 1948. Sulfonamides in the control of salmonellosis in chicks and poults. Jour. Am. Vet. Med Assn 112:296.

Tittsler, R. P.: 1932. Pullorum disease in poults. Poultry Sci. 11:78.

Van Es, L., and Olney, J. F.: 1941. Poultry diseases and parasites. Nebr. Agr. Exper. Sta., Bul. 332:42.

Wright, M. L., Anderson, G. W., Epps, N. A., and Truscott, R. B.: 1957. Further studies on pullorum disease in turkeys. Avian Dis. 1:338

SPIROCHAETOSIS

Until Hoffman *et al.* (1916) and Hoffman and Jackson (1946) reported an outbreak of spirochaetosis in turkeys in California, the disease was not known to exist in North America. No vector could be incriminated in the California outbreak although a careful search was made. Burroughs (1947) reported a case of spirochaetosis in a fowl which was produced by feeding fowl ticks (*Argas persicus*) obtained from a poultry flock in Texas. Burroughs' findings would indicate that the disease may be prevalent in chickens in Texas even though it was not previously reported. Hinshaw and McNeil (1946) studied the spirochaete obtained from Hoffman's outbreak and found it to have all the characteristics of *Borrelia anserina* (Sakharoff) (= *Spirochaeta gallinarum,* Blanchard).

Approximately a year after the outbreak referred to by Hoffman *et al.* occurred, another outbreak was diagnosed by McNeil *et al.* (1949) in a flock of adult turkeys located about 150 miles from the original outbreak. As was the case in the original outbreak, fowl ticks could not be found on the infected ranch. The spirochaetes obtained from the second outbreak were compared with those from the first outbreak and proved to be identical. Loomis (1953) reviewed the cases reported in California and attempted, without success, to transmit the disease to turkeys by means of ticks collected on ranches where the disease had been diagnosed. Other outbreaks reported in the United States include one in pheasants in California by Mathey and Siddle (1955); and in chickens in Arizona, New Mexico, and Texas by Francis (1956) and Rokey and Snell (1961). Dickie and Barrera (1964) have recently demonstrated fowl ticks in California to be carriers of *B. anserina* and capable of transmitting the disease to fowl.

Fowl spirochaetosis is widely distributed over the world. For a more complete world-wide review the student is referred to van Heelsbergen (1929), Reis and Nobrega (1957), and Lesbouyries (1941). Reviews of the literature are also given by Sreenivasan and Sankaranarayan (1945), El-Dardiry (1945), and Moreos *et al.* (1946). The only other published reference to a natural outbreak in turkeys is one by Stylianopoulos (1925) which is referred to by Lesbouyries (1941). This outbreak occurred in Greece in turkey poults. For a complete description of this disease in turkeys and its causative agent the reader is referred to McNeil *et al.* (1949). For a good general review of pathogenic spirochaetes, the reader is referred to Stavitsky (1948). For other fowl see Chapters 14 and 37.

Description of the organism. *Bergey's Manual of Determinative Bacteriology*, seventh edition (Breed *et al.*, 1957), lists *Borrelia anserina* as the accepted name of the spirochaete originally isolated from geese by Sakharoff (1891) and called by him *Spirochaeta anserina*. Other synonyms are *Sp. gallinarum, Sp. anatis,* and *Treponema anserinum*. Bergey's sixth edition (Breed, *et al.*, 1948) and Davis (1948) give a more exhaustive historical résumé of the organism than does the condensed seventh edition of *Bergey's Manual*. Sakharoff (1891) first described the organism from the

blood of geese suffering from a severe febrile disease in the Caucasus. His original description includes a photomicrograph which shows about six spirals, but does not give measurements of length. Reports in the literature of the length have varied from 6 to 30μ, and there is wide variation in the same bird, due to division stages. Hinshaw and McNeil (1946) reported an average of 14μ (7 to 21μ) with six spirals (Fig. 41.33). The organism is motile, stains readily with aniline dyes (in contrast to Leptospira and Treponema), and is soluble in 10 per cent ox bile and 10 per cent saponin. At crisis the spirochaetes are in large clumps and are often granular (Fig. 41.34). *Borrelia anserina* also differs from Treponema in having loose, rather than tight, coils and in the ease with which it can be stained. DeLamater and Saurino (1952) compared *Treponema pallidum* after various methods of antigenic extraction and found little if any antigenic relationship. It differs from Leptospira in having looser coils and in the absence of a terminal hook as well as in ease of staining.

It should be emphasized that the spirochaete referred to by Steinhaus and Hughes (1947) is not *Borrelia anserina*. The form they described is smaller and more tightly coiled and was found in eggs infected with chicken liver tissue. It was nonpathogenic for chickens, guinea pigs, and mice. Likewise the spirochaetes described by Mathey and Zander (1955) as being associated with cecal nodules in chickens are in all probability the same as described by Steinhaus and Hughes.

Vectors. Although the fowl tick *Argas persicus* is generally referred to as the vector for *Borrelia anserina*, it is by no means the only one. Others that have been reported include the common red mite *Dermanyssus gallinae* by Hungerford and Hart (1937), and Culex mosquitoes by Zuelzer (1936). Direct transmission is also possible by several routes including oral, intranasal, intraorbital, intravenous, and subcutaneous. Kapur (1940) was able to transmit the disease in chickens by smearing the infected material on the unbroken skin of the comb or the breast. The incubation period in such cases was 2 to 6 days.

Signs. Listlessness, cyanosis of the head, increased thirst, fever, and yellowish-green diarrhea with increased urates are characteristic. The area around the vent
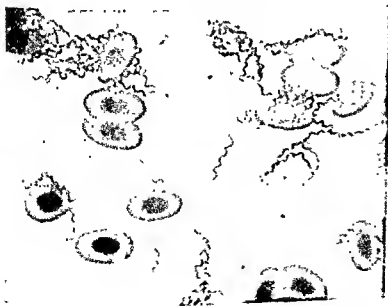
FIG. 41.33 — Borrelia anserina in turkey blood. This smear was made in the early stages of the disease. ×1,200. (McNeil et al., 1949.)

FIG. 41.34 – *Borrelia anserina*, showing clumping in the late stage of the disease. ×1,200. (McNeil et al., 1949.)

is nearly always stained with urates. In turkeys artificially infected by the intravenous route, the body temperature usually increases within 24 hours following infection and reaches a peak of 109.0° to 111.0° F. on the fourth or fifth day. By the end of the seventh or eighth day, if the bird lives, the temperature usually returns to normal. In naturally developed cases, temperatures as high as 109.4° F. have been observed. Infected birds tend to sit with their eyes closed unless disturbed. Chronically affected individuals develop leg weakness and sit characteristically on their hocks (Fig. 41.35). When disturbed they move about by hopping, often in a semisquatting position rather than on their feet. Others walk with a stilted gait. Complete paralysis has occasionally been noted.

Necropsy findings. The most characteristic gross change noted at necropsy is a marked enlargement and mottling of the spleen, due to ecchymotic hemorrhages such as are seen in Figure 41.36. The heart may be enlarged and have a parboiled appearance. The liver is usually enlarged, congested, and more or less studded with minute areas of necrosis. In advanced cases the areas of necrosis may be as much as a centimeter in diameter. Peripheral infarcts reported in chickens have not been a common finding in turkeys. The kidneys are enlarged and, as a rule, slightly pale. The intestines appear anemic when superficially examined. There is always a marked catarrhal enteritis, and the contents are bile stained. The increase in urates is noted by the abnormal amount in the rectum; these are yellowish-green in color.

Histopathology. McNeil et al. (1949) give a detailed description of the microscopic tissue changes observed by them. The spleen presented the most characteristic changes macroscopically (Fig. 41.36) as well as microscopically (Fig. 41.37). The characteristic structure of the spleen is not
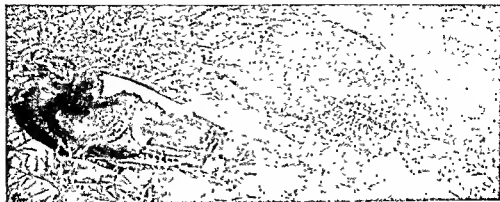
FIG. 41.35 — Spirochaetosis. Typical altitudes seen in acute outbreaks in turkey flocks. (Hinshaw.)

lost. The reticular cells are increased in number and size and present a foamy appearance due to the ingestion of lipoid material. The centers of the groups of reticular cells undergo hyalinization, and massive areas of hemorrhage are present (Fig. 41.37) due to the rupture of the walls of veins and sinusoids. The endothelial cells lining these structures appear swollen and present the foamy appearance seen in the primitive reticular cells. The diffuse lymphatic tissue undergoes rapid growth. The cells consist of young, large- and medium-sized lymphocytes and hemocytoblasts with numerous mitotic figures. In poults spirochaetes occur in foci throughout the spleen but do not appear to have been phagocytized by the reticular cells.

The liver shows congestion and an increase in the periportal lymphoid deposits. Silver stains show the majority of the spirochaetes to be in the intercellular spaces and in the bile capillaries. Those within the hepatic cells underwent fragmentation or coiled upon themselves to form small rings.

The kidneys show marked congestion and some hemorrhage. The glomeruli and convoluted tubules appear normal. There is marked degeneration and desquamation, with hyaline casts in the collecting tubules. The interstitial tissue shows lymphocytic infiltration. No spirochaetes have been found in the kidneys of the adults outside of the larger blood vessels. In poults spirochaetes were found in the intercellular
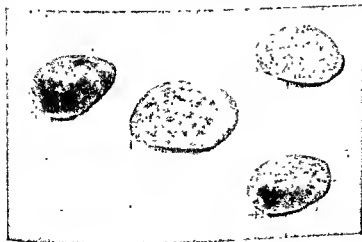
FIG. 41.36 — Spirochaetosis. Spleens from adult turkey hens, showing the typical mottling and ecchymosis. Approximately three-fourths normal size. (Hinshaw.)
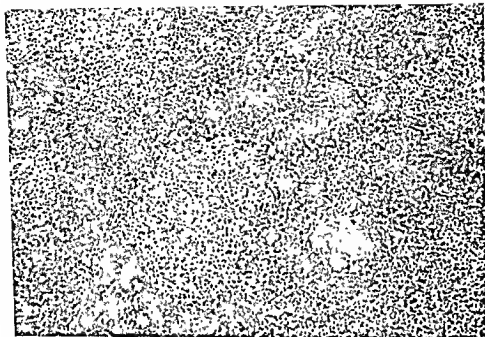
FIG 41 37 — Section of spleen showing massive areas of hemorrhage. ×150. (McNeil et al., 1949.)

spaces and in the lumens of the tubules.

The intestines show a marked degree of catarrhal enteritis The lymphoid follicles in the submucosa are hypertrophied, and there is a generalized lymphocytic infiltration of the submucosa. The tips of some of the villi appear necrotic. The most severe injury occurs in the jejunum. The pancreas appears normal except for slight vacuolization of the secreting cells.

Diagnosis. An accurate diagnosis depends on finding spirochaetes in stained blood smears and tissues from typically sick individuals (Figs. 41.33 and 41.34). The organisms are readily stained by the Giemsa technique. Tunnicliff's technique for using her modified Gram's stain for spirochaetes is a simple and rapid method for staining blood smears for routine examinations. In tissues it is best demonstrated by some method of silver impregnation such as that of Levaditi or by the slow method of Giemsa.

A method of diagnosing the disease in dead birds, where it is impossible to demonstrate the parasites, is described by Nobrega and Reis (1947). The method is based on demonstration of antibodies in the spleen and is briefly as follows:

The spleen is weighed and a 30 per cent suspension of it is made in physiological saline to which is added 10 per cent fresh rabbit serum. To each 1 cc. of this mixture is added 0.25 cc. of a 1:10 dilution of known infected blood. The samples are incubated for 1.5 hours at 37° C. and then inoculated intramuscularly into 1- to 2-month-old cockerels. A parallel series is inoculated with spleen suspensions only and others with infected blood only. The blood of all birds is examined on the fourth day after inoculation. Failure of the cockerels in the first group to develop spirochaetosis indicates that the dead bird donor had the disease and had developed enough antibodies to prevent infection. The authors claim an 85 per cent increase in efficiency in diagnosis in dead birds by this technique.

B. anserina is not easily cultivated in vitro. For a discussion of the methods of culture see McNeil et al. (1949). Many workers prefer to maintain the spirochaetes in the tick Argas persicus, but one hesi-

tates to use this method in an area where the disease is not enzootic. Knowles *et al.* (1932) were able to grow them in embryonated but not in infertile eggs. In embryos the infection usually kills the embryo. McNeil *et al.* (1949) were able to cultivate them in both chicken- and turkey-embryonated eggs, but, under conditions available at the time, found it more feasible to maintain cultures by passage in chicks at 5-day intervals. McKercher (1950) successfully propagated the organisms in chicken embryos by a modification of the Knowles *et al.* method.

McNeil *et al.* (1949) found that the organisms remained alive in tissues of infected chicks and poults when stored at 32° F. Tissues (spleen, heart, and liver) stored at this temperature for as long as 31 days were infective when injected intraperitoneally into young chicks and poults. Infected blood stored at 20° F. for 8 days was still infective. McKercher (1950) found that infected chorio-allantoic fluid stored at 39° F. (4° C.) remained infective for 2 to 3 weeks.

Prevention and control. Since this disease is normally transmitted by such vectors as fowl ticks, mosquitoes, and fowl lice, a program for eradication of these will do much to prevent the spread of the disease. DDT has been found to be effective against fowl ticks. Drugs that have proven to be effective for the disease in chickens include the arsenicals (Moreos *et al.*, 1946) and penicillin (Nobrega and Bueno, 1945). McNeil *et al.* (1949) confirmed the findings of Nobrega and Bueno and indicate that a single dose of 10,000 to 15,000 units of penicillin given intramuscularly to mature turkeys is highly effective as treatment if given when clinical signs are first noted. A single dose of penicillin injected into the breast muscles at this time has proved an effective method of controlling an outbreak. Sick birds should be removed as soon as observed and treat-

ed immediately, after which they should be kept separately from the remainder of the flock for observation for a few days. A few birds may have to be retreated but the majority recover with one treatment. Recovered birds treated after the spirochaetes had invaded the tissues (showing symptoms) were found to be immune. Sulfonamides and streptomycin were not effective in controlling the disease. Neoarsphenamine (10 mg per kilogram) and mapharsen (5 mg per kilogram) were no more effective than penicillin.

Packchanian (1950) and Hsiang and Packchanian (1951) used 2- to 4-day-old chicks for studying the effectiveness of a number of antibiotics and drugs. Terramycin in single doses of 0.1 mg. per 50 gm. was the most effective of the antibiotics tried in young chicks. Others that were effective, but less so, included Aureomycin, bacitracin, penicillin-G, and streptomycin. Of the other drugs tried, neoarsphenamine in a single dose of 1.0 mg. was the most effective. Turkeys were not used in these trials.

Nobrega and Reis (1941) reported the successful use of a formalized vaccine prepared from infected chicken embryos for prevention of the disease. Their vaccine is prepared by inoculation of 12-day-old embryos with 0.05 to 1.0 cc. of chicken blood containing live spirochaetes. On the fifth day of incubation after infection, the organs of the embryo with the amniotic fluid are ground together and suspended in saline to make 30 cc. for each egg. Formalin is added to make 0.5 per cent. and the suspension is then left in the refrigerator for 24 hours before being filtered through sterile gauze. This "stock suspension" is diluted with 3 parts of saline for use, and 1.0 cc. is injected intramuscularly in each bird. A modification of the method of Nobrega and Reis has been described and used successfully by Rao *et al.* (1954).

REFERENCES

Breed, R. S., Murray, E. G. D., and Hitchens, A. P.: 1948. Bergey's Manual of Determinative Bacteriology. 6th ed. Williams and Wilkins Co., Baltimore. 1529 pp.
———, Murray, E. G. D., and Smith, N. R.: 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams and Wilkins Co., Baltimore. 1094 pp.

Burroughs, A. L.: 1947. Fowl spirochetosis transmitted by *Argas persicus* (Oken), 1818 from Texas. Science 105:577.

Davis, G. E.: 1948. The spirochetes. Ann. Rev. Microbiol. 2:305.

DeLamater, E. D., and Saurino, V. R.: 1952. Studies on the immunology of spirochetes. Bact. Proc., 52nd Gen. Meet. Soc. Am. Bact. Boston, Mass. P 127.

Dickie, C. W., and Barrera, J.: 1964. A study of the carrier state of avian spirochetosis in the chicken. Avian Dis. 8:191.

El-Dardiry, A. H.: 1945. Studies on avian spirochetosis in Egypt. Ministry of Egypt, Tech. Sci. Service Bul 243:1.

Francis, D. W.: 1956. A case of fowl spirochetosis in New Mexico. Poultry Sci. 35:1142.

Hinshaw, W. R., and McNeil, E.: 1946. Studies on a spirochaete found in the blood of sick turkeys. Jour. Bact. 51:599.

Hoffman, H. A., and Jackson, T. W.: 1946. Spirochetosis in turkeys. Jour. Am. Vet. Med. Assn. 109:481.

——, Jackson, T. W., and Rucker, L. C.: 1946. Spirochetosis in turkeys (a preliminary report). Jour. Am. Vet. Med. Assn. 108:329.

Hsiang, Chin-Min, and Packchanian, A.: 1951. A comparison of eleven antibiotics in the treatment of *Borrelia anserina* infection (spirochetosis) in young chicks. Texas Repts. Biol. and Med. 9:54.

Hungerford, T. G., and Hart, L.: 1937. Fowl tick fever (spirochaetosis), also transmitted by common red mite Agr. Gaz New So. Wales 48:591.

Kapur, H R.: 1940. Transmission of spirochaetous through agents other than *Argas persicus*. Indian Jour Vet Sci. and Anim Husb. 10:354.

Knowles, R., Das Gupta, B. M., and Basu, B. C.: 1932. Studies in avian spirochaetosis. Indian Med. Res. Mem. 22:1.

Lesbouyries, G: 1941. La Pathologie des Oiseaux. Vigot Frères, Paris.

Loomis, E. C.: 1953 Avian spirochetosis in California turkeys. Am. Jour. Vet. Res. 14:612.

McKercher, D. G.: 1950 The propagation of *Borrelia anserina* in embryonated eggs employing the yolk sac technique. Jour. Bact. 59:466.

McNeil, E., Hinshaw, W. R., and Kissling, R. E.: 1949. A study of *Borrelia anserina* infection (spirochetosis) in turkeys. Jour. Bact. 57:191.

Mathey, W J., and Siddle, P J : 1955. Spirochetosis in pheasants. Jour. Am. Vet. Med. Assn. 126 123

——, and Zander, D. V : 1955. Spirochetes and cecal nodules in poultry. Jour. Am. Vet. Med. Assn. 126:475.

Morcos, Z., Zaki, O. A., and Zaki, R.: 1946. A concise investigation of fowl sprochaetosis in Egypt. Jour. Am. Vet. Med. Assn. 109:112.

Nobrega, P., and Bueno, R. C.: 1945. A accao da penicilina na espiroquetose aviaria. Arq. Inst. Biol. São Paulo 16:15.

——, and Reis, J.: 1941. Producao da vacina contra a espiroquetose aviaria em ovos embrionados. Arq. Inst. Biol São Paulo 12:87.

——, and Reis, A S : 1947 O diagnóstico da espiroquetose aviaria em animais mortos. Arq. Inst Biol. São Paulo 18:91.

Packchanian, A : 1950 Chemotherapy of *Borrelia anserina* infections (spirochetosis) in young chicks. Texas Repts. Biol and Med 8:78.

Rao, S. B. V., Thakral, B. M., and Dhanda, M R.: 1954. Studies on fowl spirochaetosis with special reference to penicillin therapy and the development of an egg-adapted vaccine for its control. Indian Vet. Jour. 31:1.

Reis, J, and Nobrega, P.: 1957. Tratado de Doencas das Aves, 2nd Ed. Inst. Biol São Paulo, Brazil.

Rokey, N. W., and Snell, V. N.: 1961. Avian spirochetosis (*Borrelia anserina*) epizootic in Arizona poultry. Jour. Am. Vet. Med. Assn. 138 648

Sakharoff, M. N.: 1891. Spirochaeta anserina et la septicemie des oies. Ann. Inst. Pasteur 5:564.

Sreenivasan, M. K., and Sankararamayan, N. S : 1945. Spirochaetosis of fowls in India. Indian Vet. Jour. 21:325.

Stavitsky, A. B.: 1948. Characteristics of pathogenic spirochetes and spirochetoses with special reference to the mechanisms of host resistance. Bact. Revs. 12:203.

Steinhaus, E. A., and Hughes, L. E.: 1947. Isolation of an unidentified spirochete from hen's eggs after inoculation with liver tissue from hens. U.S. Pub. Health Rept 62:309.

Styllanopoulos: 1925. (Original not seen, quoted by Lesbouyries, 1941)

van Heelsbergen, T.: 1929. Handbuch der Geflügelkrankheiten und der Geflügelzucht. Ferdinand Enke, Stuttgart.

Zuelzer, M.: 1936. *Culex*, a new vector of *Spirachaeta gallinarum*. Jour. Trop. Med. and Hyg. (London) 39.204.

STAPHYLOCOCCOSIS (Staphylococcal Synovitis, Staphylococcal Arthritis)

Jungherr (1933) and Jungherr and Plastridge (1941) reported this disease and similar ones in poultry under the name "staphylococcosis," and reviewed the literature to those dates. Madsen (1942) described a similar disease characterized by

synovitis without involvement of the articular joints. Hinshaw and McNeil (1952) reported field observations on the disease in turkeys and also reported results on experimental production of the disease in turkeys and chickens. The causative organism studied by them is a strain of *Staphylococcus aureus* which has a characteristic biochemical reaction. It ferments dextrose, lactose, maltose, mannitol, xylose, cellobiose, dulcitol, and salicin. It is indol negative, liquefies gelatin, reduces nitrates to nitrites and is Voges-Proskauer and methyl-red positive. The individual strains varied in their ability to coagulate turkey plasma. The pigment produced varied from cream to yellow in color. Of the media tested, beef heart infusion agar or beef heart infusion broth gave best results. Primary isolation was more successful in broth than on solid medium.

The Utah Agricultural Experiment Station has probably done the most intensive research on this disease in recent years (Miner, 1957; Smith *et al.*, 1961a). Smith *et al.* (1961a) obtained 9 new staphylophages and a previously described one (44A) from 27 staphylococcal cultures isolated from 23 turkeys, 2 mice caught in a turkey yard, and one sample of turkey yard soil. Based on their studies it would appear that several phage types may be involved in outbreaks of the disease in turkeys. All turkey strains studied produced alpha-type hemolysin, fermented mannitol, utilized gelatin, and were positive for coagulase.

Fahey (1954) described an outbreak in poults in Canada, and Smith (1954a, b) reported experimental production of the disease in chickens. Other references to staphylococcus infections in birds will be found in Chapter 16.

According to Smart and Miner (1961) staphylococcosis can be seen in turkeys from 3 weeks of age to maturity. Poults have yielded the organism before they leave the hatchery at 2 days of age (Smith *et al.*, 1961b) even though clinical illness and death may not come for many weeks. This could suggest egg transmission or even transmission by human carriers handling poults during the hatching operations. Phage typing of strains of *Staph. aureus* isolated from hatchery operators and poults in the same hatchery would aid in locating sources of infection. The possibility of man to poult or poult to man transmission is suggested by the report of Williams and Daines (1942) on the relationship of staphylococcal omphalitis in poults to impetigo staphylogenes among turkey hatchery workers.

Signs. It should be emphasized that in turkeys the disease is first of all a septicemia with later localization in the tibiometatarsal and femorotibial joints, in the feet, and in sternal abscesses.

The acute symptoms are similar to those of such diseases as fowl cholera. In artificially produced cases the birds become listless within 24 to 72 hours. This is accompanied by loss of appetite, loss in weight, and an increase in temperature to 109.0° F. Within 2 to 5 days the feces become fetid and sulfur-colored and the disease could be mistaken for histomoniasis. The birds seem to suffer great pain and cry out when handled or forced to walk. Paralysis occurs but not as often as in chickens artificially infected. Swelling of the feet and hock joints appears 48 to 96 hours after intravenous inoculation (Figs. 41.38 and 41.39). Sternal abscesses, which in the field outbreaks are often considered the primary lesion, developed in these cases as early as the swollen joints.

Necropsy findings and diagnosis. Necropsy of acute cases reveals an enlarged

FIG. 41.38 — Staphylococcal arthritis in young poult. (Hinshaw, Univ. of Calif.)

FIG. 41.39 — (A) Staphylococcal arthritis in an adult turkey. Note swollen joints of the feet. (B) Close-up of the feet of the turkey shown in A. (Hinshaw, Univ. of Calif.)

A   B
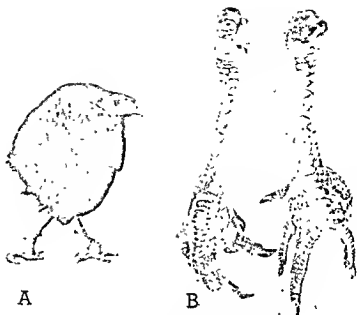
and dark liver and congestion of the mucous membranes of the intestines. The intestinal contents are watery and yellowish in color Inflammation of the synovial membranes of the hock joints, with increased fluid, is characteristic. Chronic cases show, principally, involvement of the joints and muscles of the legs and feet The exudate may vary from a semigelatinous to a cheeselike flaky consistency. One of the common lesions is a sternal abscess. Often this is a sac pustule 2–3 inches long and 1 inch wide, filled with a yellow purulent to a caseous exudate. Occasionally abscesses between the pectoral muscles are seen. Fibrinous pericarditis has been observed in acute cases.

Diagnosis depends on isolation of the causative organism. The disease must be differentiated from other types of joint inflammations. Scott (1950, 1951) described a deformity in poults characterized by swelling of the tibiometatarsal joint which is associated with a failure in retention of creatinine. This disease is a different entity but could predispose turkeys to staphylococcosis. Other dietary disturbances affecting the joints could likewise influence the effect of the disease in

a flock. The disease must also be differentiated from infectious synovitis which is caused by a viruslike agent affecting both chickens and turkeys (Cover and Benton, 1957).

Control and prevention. Hinshaw and McNeil (1952) tried penicillin, sulfanilamide, sulfathiazole, sulfamerazine, and suffamethazine in field outbreaks without success. They also observed that inoculated turkeys surviving the infection were not immune when reinoculated at a later date. The strains isolated from outbreaks by them were resistant to penicillin when tested in vitro. Of interest in this connection was that a penicillin-resistant strain isolated from a mastitis outbreak in cattle produced the typical disease in turkeys. Fahey (1954) and Miner et al. (1958) also found that most of the strains isolated by them from outbreaks were penicillin-resistant. Fahey (1955) stated that in uncomplicated outbreaks, 300 gm. of a mixture (100 gm. each) of streptomycin, Terramycin, and Aureomycin given in feed for 1 week yielded good results.

Miner et al. (1958) and Smart and Miner (1961) found that the antibiotic novobiocin has promise of being a successful drug for

treatment of the disease. The drug was given at the rate of 200 to 600 grams of feed grade of novobiocin per ton of feed starting soon after the disease appeared in a flock. These investigators found that furazolidone was of little if any value in controlling the disease. It appears, on the basis of present knowledge, that broad-spectrum antibiotics have the most merit

in controlling field outbreaks of this disease. *The drug of choice for a specific outbreak should be ascertained by an early determination of the antibiotic sensitivity of the strain of Staphylococcus responsible for the outbreak.*

The general recommendations for handling other infectious diseases are suggested.

REFERENCES

Cover, M. S., and Benton, W. J.: 1957. The distribution of the infectious synovitis agent in the tissues of artificially infected chickens. Avian Dis 1:312

Fahey, J. E.: 1954. An outbreak of staphylococcal arthritis in turkey poults. Poultry Sci. 33:661

——: 1955. University of Toronto, personal communication

Hinshaw, W. R., and McNeil, E.: 1952. Staphylococcus (synovitis) in turkeys. Poultry Sci. 31:320

Jungherr, E.: 1933. Staphylococcal arthritis in turkeys. Jour. Am. Vet. Med. Assn. 82:243.

——, and Plastridge, W. M.: 1941. Avian staphylococcosis. Jour. Am. Vet. Med. Assn. 98:27.

Madsen, D. E.: 1942. Synovitis of turkeys. Turkey World 17:24.

Miner, M. L.: 1957 (June). Staphylococcal synovitis of turkeys   Norbest Turkey News 21:1.

——, Smart, R. A., and Smith, W. W.: 1958. The use of nitrofurans and antibiotic in staphylococcis of turkeys. Proceedings, 2nd National Symposium on Nitrofurans in Agriculture: 135.

Scott, M. L.: 1950. Studies on the enlarged hock disorder (perosis) in turkeys. Jour. Nutr. 40:611

——: 1951. Studies on the enlarged hock disorder in turkeys. 2. Factors affecting the excretion and retention of creatine by young poults. Poultry Sci. 30:839.

Smart, R. A., and Miner, M. L.: 1961. Treatment of staphylococcal synovitis of turkeys. Poultry Sci. 40:676.

Smith, H. W.: 1954a. The pathogenicity and haemolytic properties of staphylococci isolated from chickens. Jour. Path. and Bact. (Brit.) 67:73.

——: 1954b. Experimental staphylococcal infection in chickens. Jour. Path. and Bact (Brit.) 67:81.

Smith, W. W., James, G. A., Miner, M. L., Bloomer, E. F., and Jensen, M. L.: 1961a. A phage-typing system for Staphylococci from turkeys with synovitis. Am. Jour. Vet. Res. 22:588.

——, Miner, M. L., Thomas, J. A., Carter, P. B., and Elsner, Y. Y.: 1961b. Evidence of late transmission of Staphylococcus aureus strains within a divided flock of turkeys after 11 weeks of age. Am. Jour. Vet. Res. 22:753.

Williams, R. B., and Daines, L. L.: 1942. The relationship of infectious omphalitis of poults and impetigo staphylogenes in man. Jour. Am. Vet. Med. Assn. 101:26.

STREPTOCOCCOSIS

Volkmar (1932) reported several outbreaks of apoplectiform septicemia in turkeys caused by a streptococcus, and described the disease as resembling fowl cholera. The losses were sporadic in nature, the disease was acute, and clinical signs were seldom seen before death. The principal lesions noted on necropsy were congestion or diffuse hemorrhages in the skin and breast muscles, together with generalized congestion of the internal organs. Hemorrhagic enteritis and peritonitis were common, and the heart sac was often filled with a blood-tinged fluid. The disease must be differentiated by bacteriologic studies.

Acute outbreaks of a disease in young

poults with signs and necropsy findings resembling pullorum disease were found by McNeil and Hinshaw in California (unpublished data). Losses in these cases have equalled those of pullorum disease or paratyphoid infections. Examples of mortality experienced in these outbreaks are: 430 out of 1,080 poults; 500 out of 1,200 poults; and 450 out of 1,500 poults. Losses started within the first week and continued for two weeks. The signs resembled those of pullorum disease. Necropsy findings included congestion and necrosis of the liver, lungs, congestion and necrosis of the liver, and enteritis. Pin-point areas of necrosis in the livers were especially common. A short-chain streptococcus which has the

characteristics of *Streptococcus zymogenes* was consistently isolated from these cases. No detailed studies have been made of this disease, but mention is made of it

since it may be confused with pullorum disease. Other references to avian streptococcosis will be found in Chapter 16.

REFERENCE

Volkmar, F : 1932 Apoplectiform septicemia in turkeys Poultry Sci. 11:297.

TUBERCULOSIS*

Tuberculosis, a chronic disease affecting turkeys and other fowls, is caused by *Mycobacterium avium*. It is not common in commercial turkey flocks and is most often associated with tuberculous chickens.

Signs. There are no typical clinical signs. Lameness and emaciation have occasionally been observed Many turkeys that show lesions on necropsy maintained their weight for several months before death Tuberculous turkeys placed in individual cages and observed for periods of 1 to 10 weeks held their initial weight, and a few even gained Such birds often went through intermittent periods of normality and depression lasting for 2 or 3 weeks before death.

Clinical diagnosis. Tuberculosis in turkeys flocks has been more often detected by accidental discovery of lesions during postmortem examination by the owner, or by the housewife while preparing a bird for roasting, than by signs seen in the flock or by the use of the tuberculin test. Hinshaw *et al.* (1932) found that about 75 per cent efficiency can be expected from the use of the tuberculin test as a means of diagnosis. The edge of the wing web proved to be the best site for inoculation of the tuberculin, but the results, even in this area, were more difficult to interpret than in other animals

Necropsy findings. The gross pathology of tuberculosis in turkeys is not markedly different from that of the disease in chickens. The distribution of lesions indicates a tendency for a greater number of organs to become infected than in chickens, and, as in chickens, the disease is principally abdominal in nature. Seven cases of tuberculosis in turkeys from five California

outbreaks were typed and found to be of avian origin.

A study of the distribution of lesions in turkeys from seven California outbreaks showed that the liver, bone marrow, spleen, intestines, ovaries, mesentery, skin, thymus gland, and lungs were, in the order given, the most common sites of lesions. Cutaneous lesions in turkeys have also been reported by Scrivner and Elder (1931) and according to Feldman (Chapter 12) by Chrétien *et al.* (1925) and Dietrich (1927). The ovary and the thymus glands were more often found to be infected than in chickens. Attention is also called to the large percentage of cases of bonemarrow lesions. The number of birds examined for bone-marrow lesions was small as compared to the total, but they were in all stages of the disease. When lesions were found in the bone marrow, they were always found in at least one other organ.

Differential diagnosis. Some of the conditions which might be confused with tuberculosis in turkeys are mycosis, blackhead, and tumors. Mycotic lesions in the liver and kidney, which on first glance are suggestive of tubercles, have been observed. These are not definitely encapsulated and circumscribed, however. On microscopic examination, mycelia are found, while acid-fast rods cannot be demonstrated.

Infectious enterohepatitis should not be confused with tuberculosis, because the lesions in the liver do not resemble tubercles. Furthermore, the well-known characteristic lesions in the ceca should help to differentiate it from tuberculosis. On the other hand, tumors of the liver and ovary have been noted that were suggestive of tuberculosis until a microscopic examination was made.

Prevention and control. Complete isolation of turkeys from chickens will do much

* See also Chapter 12.

to prevent tuberculosis. Once the disease is found, it is a good plan to dispose of the entire flock as well as all chickens on the premises.

Day-old poults, rather than adult stock, should be purchased as replacements. They should be brooded away from the infected area and should not be allowed to range there for at least one year after the disposal of diseased birds. The entire flock should be sold and replaced with day-old stock each spring for several years in order to insure freedom from tuberculosis.

REFERENCES

Chrétien, A., Germain, and Raymond: 1923. Anatomie pathologique de la tuberculose aviaire. Rev. de la tuberc. 4:25. (Quoted by Feldman, Chapter 12)

Dietrich, A.: 1927. Ein Fall von Hauttuberguose bei einer Pute. Berliner tierärtzl. Wochenschr. 43:294. (Quoted by Feldman, Chapter 12.)

Hinshaw, W. R., Niemann, K. W., and Busic, W. H.: 1932. Studies of tuberculosis of turkeys. Jour. Am. Vet. Med. Assn. 80 765.

Scrivner, L. H., and Elder, C.: 1931. Cutaneous and subcutaneous tuberculosis in turkeys. Jour. Am. Vet. Med. Assn. 79:244.

TRANSMISSIBLE ENTERITIS (Avian Monocytosis, Bluecomb Disease)

This disease is described under various names, including avian monocytosis and bluecomb disease, because of characteristics in common with the disease of chickens known under these names. Transmissible enteritis has been suggested by Sieburth and Johnson (1957) as the most appropriate name for the disease of turkeys. It has also been confused with hexamitiasis because of many characteristics common to the two diseases. It is now generally accepted that the cause is a filterable agent (Sieburth and Johnson, 1957; Tumlin et al., 1957; Tumlin and Pomeroy, 1958; Truscott et al., 1960; and Truscott and Morin, 1963). Truscott and Morin have isolated a Vibrio sp. which they believe to be an etiological factor in production of the disease. Pomeroy (personal interview, 1964) reported that he and his colleagues (unpublished data) have also isolated a Vibrio sp. as well as a viral agent from field outbreaks. The true etiological significance of these microorganisms remains to be determined.

The disease affects susceptible turkeys of all ages, without regard to season, especially on farms that practice a continuous rearing program. In young poults the first signs of the disease may appear as early as the fourth or fifth day after exposure. They are very similar to those seen in hexamitiasis and include listlessness, tendency to seek heat, subnormal temperature, watery droppings, and rapid loss of weight. Since these are also characteristic symptoms of hexamitiasis, the two diseases must be differentiated by demonstration of their respective etiological agents. Because of the similar age range of susceptibility, it is highly probable that combined infections may occur.

Because of the similarity to avian monocytosis of chickens, the reader is referred to Chapter 31, Avian Monocytosis, for complete information on the etiology, pathology, prevention, and control of that disease.

REFERENCES

Sieburth, J. McN., and Johnson, E. P.: 1957. Transmissible enteritis of turkeys (bluecomb disease). I. Preliminary studies. Poultry Sci. 36:256.

Truscott, R. B., Connell, M. C., Ferguson, A. E., and Wills, C. G.: 1960. A bacterial agent causing bluecomb disease in turkeys. I. Isolation and preliminary laboratory investigations. Avian Dis. 4:391.

———, and Morin, E. W.: 1963. A bacterial agent causing bluecomb disease in turkeys. II. Transmission and studies of the etiological agent. Proc. 35th N.E. Conf. on Avian Dis. Amherst, Mass. June 17-19, 1963.

Tumlin, J. T., and Pomeroy, B. S.: 1958. Bluecomb disease of turkeys. V. Preliminary studies on parental immunity and serum neutralization. Am. Jour. Vet. Res. 19.725.

———, Pomeroy, B. S., and Lindorfer, R. K.: 1957. Bluecomb disease of turkeys. IV. Demonstration of a filterable agent. Jour. Am. Vet. Med. Assn. 130.560.

VIRAL HEPATITIS

Viral hepatitis of turkeys was first described simultaneously by Snoeyenbos et al. (1959) from Massachusetts, and by Mongeau et al. (1959) from Ontario, Canada. Both outbreaks occurred in poults under three weeks of age. In neither instance was the mortality excessive. The poults in the Massachusetts outbreak were 17 days of age, and the mortality was less than 5 per cent of the group of 800 birds which returned to normal appearance in 3 or 4 days. The principal clinical sign was a slight depression of activity. In another report Snoeyenbos and Basch (1960) reported the disease in 11 outbreaks in poults under 5 weeks of age with mortality rates of 1 to 25 per cent.

They also reported two cases in 6- and 8-week-old poults, and 4 diagnoses in mature stock from specimens taken at the time of slaughter. In these cases, liver condemnations of 30 to 90 per cent were recorded by the processors. Adult turkey breeder hens were infected by Snoeyenbos and Basch by intravenous injections of the virus. These birds remained clinically normal. However, at necropsy, representative birds sacrificed 2 to 16 days postinoculation yielded virus from all the livers and less frequently from other organs. In one instance, the virus was isolated from retrogressing ovarian follicular contents.

Some immunity developed in the artificially infected birds as determined by reexposure of recovered poults. Egg transmission has been suggested as one means of dissemination. No specific methods of prevention or control have been reported and no treatment is available.

Virus hepatitis as described herein must not be confused with avian vibrionic hepatitis described by Hofstad et al. (1958) and Peckham (1958). The disease described by them and others is caused by a vibrio. Peckham was able to infect turkey poults with his strain of vibrio but as far as is known there have been no reports of naturally occurring outbreaks in turkeys. A report on vibrionic hepatitis is given by Snoeyenbos in Chapter 14.

REFERENCES

Hofstad, M. S., McGehee, E. H., and Bennett, P. C.: 1958. Avian infectious hepatitis. Avian Dis 2 358.

Mongeau, J. D., Truscott, R. B., Ferguson, A. E., and Connell, M. C.: 1959. Virus hepatitis in turkeys. Avian Dis. 3:388.

Peckham, M C.: 1958. Avian vibrionic hepatitis. Avian Dis. 2:348.

Snoeyenbos, G H., and Basch, H. I.: 1960. Further studies of virus hepatitis of turkeys. Avian Dis 4.477.

——, Basch, H I., and Sevoian, M.: 1959. An infectious agent producing hepatitis in turkeys. Avian Dis. 3:377.

LEUKOSIS

Although the leukosis complex is recognized as a definite economic problem by the turkey industry, there are few specific references to it in the published literature. Evidence of the susceptibility of turkeys to leukosis and of its prevalence in them is given by McKee (1963, 1964). He reported that according to the 1960–64 official records of the USDA Agricultural Marketing Service, Poultry Inspection Branch, from 0.4 to 1.2 per cent of all turkeys condemned yearly in official processing plants were culled because of leukosis. The two types of leukosis most often seen in turkey processing plants are lymphomatosis and osteopetrosis, although all types are seen.

The importance of the need for recognizing this group of diseases and for differentiation from other diseases of turkeys is emphasized by McKee et al. (1963). Problems concerned with making differential diagnoses are also discussed by them. Diseases which may confuse a diagnostician include other neoplasms, certain chronic mycotic infections (Olney, 1950), tuberculosis, and histomoniasis. Turkeys suffering from histomoniasis may show granulomatouslike liver lesions which can only be dif-

ferentiated by micro-histological examination or by demonstration of histomonads. The fact that in such instances a number of typical histomoniasis cases usually appear in the same lot of turkeys being inspected is an aid in making a diagnosis unless both diseases are present in the same flock.

No attempt has been made to give an exhaustive review of the subject in this section. The reader is referred to Chapter 19 for a detailed discussion. Pertinent references to the leukosis complex in turkeys in addition to those given above include Andrewes and Glover (1939), Simpson et al. (1957), Belding and Sanger (1961), and Helmboldt and Frazier (1962).

REFERENCES

Andrewes, C. H., and Glover, R. E.: 1939. A case of neurolymphomatosis in a turkey. Vet. Record 51:934.

Belding, R. C., and Sanger, V. L.: 1961. The isolation and propagation of a naturally occurring turkey lymphoid tumor by cellular transplants. Am. Jour. Vet. Res. 22:271.

Helmboldt, C. F., and Frazier, M. N.: 1962. Neurofibromatosis in a turkey. Jour. Am. Vet. Med. Assn. 141:1073.

McKee, G. S.: 1963. Visceral lymphomatosis of turkey. Poultry Sci. 42:1289.

———: 1964. Personal communication. Information used by permission.

———, Lucas, A. M., Dennington, E. M., and Love, F. C.: 1963. Separation of leukotic and non-leukotic lesions in turkeys on the inspection line. Avian Dis. 7.19.

Olney, J. F.: 1950. Actinomycosis—A new disease of turkeys. Vet. Med. 45:592.

Simpson, C. F., Anthony, D. W., and Young, F.: 1957. Visceral lymphomatosis in a flock of turkeys. Jour. Am. Vet. Med. Assn. 130:93.

MISCELLANEOUS INFECTIOUS DISEASES

Numerous reports of sporadic outbreaks due to miscellaneous bacteria and viruses have been made by investigators.

Pseudomonas Infections

Pseudomonas sp. are among the common contaminants of eggshells and contents and produce the so-called "green-rot" (Lorenz et al., 1952; Orel; 1959; and Hartung and Stadelman, 1963). They must be considered as potential causes of disease in hatching poults. Personal experience of the author indicates that severe brooder losses may occur especially under unfavorable environment.

In outbreaks caused by a hemolytic Pseudomonas, Stafseth (1939) and Stafseth et al. (1940) stated that there was a morbidity of 50 per cent and a low mortality. The outstanding necropsy findings are dark and often uncoagulated blood, pin-point areas of necrosis or yellowish-gray streaks in the liver, mottled spleen, and hemorrhagic enteritis. According to these investigators, the species of Pseudomonas responsible for the outbreak in turkeys differs in several respects from P. aeruginosa, a common inhabitant in fowls.

Escherichia Infections

Frequently, diagnostic laboratories report the isolation of organisms belonging to the colon-acrogenes group of bacteria from outbreaks of septicemic diseases in young poults. In similar outbreaks organisms belonging to the paracolon groups are also reported. Paracolon infections are discussed as a separate subject in Chapter 9.

Listeriosis

Listeriosis of fowl, caused by Listeria monocytogenes, is fully described in Chapter 15. Belding and Meyer (1957), Maletwitz et al. (1957), Gray (1958), and Bolin and Eveleth (1961) have summarized the literature concerning this disease in turkeys. It is not a frequently reported disease in turkeys even in an environment where it might be expected to occur. In areas where listeriosis exists in other animals, the possibility of it occurring in turkeys must, however, be considered.

Clinical signs described include depression, nervousness, diarrhea, and at times torticollis and complete paralysis. At necropsy, necrosis of the liver which is often cropsy, necrosis of the liver which is often mottled and hemorrhages of the epicardium were described. Brain lesions were not

common. Microscopically the lesions resembled those of an acute disease.

Clostridium Infections

Fenstermacher and Pomeroy (1939) described losses in breeding turkey hens associated with several species of *Clostridium*. The outbreak involved a flock of 1,000 turkey hens in which there was a low mortality. The losses occurred during the early part of the breeding season, and the investigators stated that the atria of infection were probably wounds inflicted at the time of mating. Subcutaneous edema and emphysema around the head and *thighs were the principal symptoms* reported. Injuries due to mating can be prevented by proper management. (See section on injuries and material on botulism.)

Omphalitis

Bolin et al. (1949) described a virus isolated from chicks and poults suffering from omphalitis. The disease was characterized by a high mortality during the first three weeks after hatching. Necrosis of the tissues around the navel was an outstanding lesion. The virus proved more pathogenic for poults than chicks but could be cultivated in 10- to 12 day-old embryonated chicken eggs These workers believe the virus is transmitted through the egg. Williams and Daines (1942) also isolated a strain of *Staphylococcus aureus* from omphalitis of turkey poults which they believed to be the causative agent of an outbreak studied by them. Human cases of impetigo staphylogenes were associated with the outbreak described by them.

Actinomycosis

Olney (1950) has described an actinomyceslike chronic disease of turkeys which is characterized by liver and intestinal lesions that may be mistaken for tuberculosis. The livers in these cases are usually studded with raised nodules that may reach an inch in diameter. On cutting, these are found to be caseated, capsulated, and have fimbriated processes extending into the liver tissue from the main body of the nodule. In the outbreak described by Olney, the disease was confined to three lots of 500 poults each, all 6 weeks of age, in a total flock of 9,000 poults. When the flock was put on range at 8 weeks of age, the infected lots mingled with the normal birds, and the infection spread to them. The grower estimated a total loss of 45 per cent by the end of the season. When the birds were marketed in December, 10 per cent of them showed lesions. This disease must be differentiated from tuberculosis, histomoniasis, and lymphomatosis. More study is needed to confirm its etiology.

Pseudotuberculosis

Pseudotuberculosis is a contagious disease of many animals including turkeys and is caused by *Pasteurella pseudotuberculosis*. For a detailed description and review of the literature the reader is referred to Chapter 14. Reports of the disease in turkeys include Rosenwald and Dickinson (1944), Mathey and Siddle (1954), and Kilian et al. (1962). The disease is characterized by an acute septicemia of short duration, followed by a chronic focalized infection resulting in tubercular lesions in various organs—thus the name. The cultural characteristics of the etiological agent resembles *Salmonella pullorum*. In fact, the strain isolated by Kilian et al. agglutinated Salmonella Group D, 9, 12 antiserum when tested with the live culture, but not with alcohol-treated antigen. Serum from *P. pseudotuberculosis* infected turkeys did not however agglutinate either *S. pullorum* or *S. typhimurium* antigens. The findings of these investigators emphasize the need for positively identifying such organisms isolated from turkeys in pullorum testing programs.

Others

Numerous other infectious diseases of birds may occur in turkeys. Examples that are described in other chapters include fowl plague, psittacosis, equine and avian encephalomyelitis, coli-granuloma

(Hjärre's disease), avian hepatitis, omphalitis, and infectious synovitis. Western type equine encephalomyelitis has been reported in turkeys by Woodring (1957). Spalatin et al. (1961) have reported eastern equine encephalitis in Wisconsin turkeys, and Porterfield (1961) has described a meningoencephalitis virus from turkeys in Israel.

A recent outbreak of fowl plague in turkeys is described by Wells (1963). This outbreak occurred in a flock of turkeys on the North Norfolk coast of England in the spring of 1963. The main features of the outbreak were typical of the disease and included rapid spread, sudden deaths, high mortality, and marked drop in egg production in the layers. The source was not discovered but it was thought to be introduced by migrating birds. The outbreak was eradicated by radical quarantine and slaughter methods. Within a month, 29,000 turkeys were dead from the disease or by slaughter.

REFERENCES

Belding, R. C. and Mayer, M. L.: 1957. Listeriosis in the turkey—two case reports. Jour. Am. Vet. Med. Assn. 131:296.

Bolin, F. M., and Eveleth, D. F.: 1961. Experimental listeriosis of turkeys. Avian Dis. 5:229.
——, Schlamb, K. F., Bryant, R. L. and Eveleth, D. F.: 1949. A virus disease of turkeys and chickens. Am. Jour. Vet. Res. 10:391.

Fenstermacher, R., and Pomeroy, B. S.: 1939. Clostridium infection in turkeys. Cornell Vet. 29:25.

Gray, M. L.: 1958. Listeriosis in fowls—a review. Avian Dis. 2:296.

Hartung, T. E., and Stadelman, W. J.: 1963. Pseudomonas fluorescens penetration of shell membranes as influenced by shell porosity, age of egg and degree of bacterial challenger. Poultry Sci. 42:147.

Kilian, J. G., Yamamoto, R., Babcock, W. E., and Dickinson, E. M.: 1962 An unusual aspect of Pasteurella pseudotuberculosis in turkeys. Avian Dis. 6:405.

Lorenz, F. W., Starr, P. D., Starr, M. P., and Ogasawara, F. X.: 1952. The development of Pseudomonas spoilage in shell eggs. I. Penetration through the shell. Food Res. 17:351.

Malewitz, T. D., Gray, M. L., and Smith, E. M.: 1957. Experimentally induced listeriosis in turkey poults. Poultry Sci. 36:416.

Mathey, W. J., and Siddle, P. J.: 1954. Isolation of Pasteurella pseudotuberculosis from a California turkey. Jour. Am. Vet. Med. Assn. 125:482.

Olney, J. F.: 1950. Actinomycosis—A new disease of turkeys. Vet. Med. 45:392.

Orel, V.: 1959. The Pseudomonas spoilage of eggs laid by individual hena. Poultry Sci. 38:8.

Porterfield, J. S.: 1961. Israel turkey meningo-encephalitis virus. Vet. Record 73:392.

Rosenwald, A. S., and Dickinson, E. M.: 1944. A report on Pasteurella pseudotuberculosis infection in turkeys. Am. Jour. Res. 5:246.

Simpson, C. F., Anthony, D. W., and Young, F.: 1957. Visceral lymphomatosis in a flock of turkeys. Jour. Am. Vet. Med. Assn. 130:93.

Spalatin, J., Karstad, L., Anderson, J. R., Lauerman, L., and Hanson, R. P.: 1961. Natural and experimental infections in Wisconsin turkeys with the virus of eastern encephalitis. Zoonoses Res. 1:29.

Stafseth, H. J.: 1939. Pseudomonas infection in turkeys. Poultry Sci. 18:412.
——, Mack, W., and Ryff, J. F.: 1940. Pseudomonas infection in turkeys. Poultry Sci. 19:126.

Wells, R. J. H.: 1963. An outbreak of fowl plague in turkeys. Vet. Record 75:783.

Williams, R. B., and Dames, L. L.: 1942. The relationship of infectious omphalitis of poults and impetigo staphylogenes in man. Jour. Am. Vet. Med. Assn. 101:26.

Woodring, F. R.: 1957. Naturally occurring infection with equine encephalomyelitis virus in turkeys. Jour. Am. Vet. Med. Assn. 130:511.

Protozoan Diseases*

HISTOMONIASIS (Infectious Enterohepatitis, Blackhead)

This disease, first described by Cushman (1893), is caused by a protozoan parasite, Histomonas meleagridis. It is credited with being the cause of the temporary abandonment of the turkey industry in some sections of eastern and mid-western United States. The early researches of Smith (1895), Moore (1896), Curtice (1907), Higgins (1915), Smith and Graybill (1920), and Tyzzer (1920) paved the

* See also Chapter 37.

way for later studies which proved that the disease is preventable.

Etiology. *Histomonas meleagridis*, the causative agent, is classified as a flagellate but is one of the few that likewise has an amoeboid stage. It is harbored by the common poultry cecal worm, *Heterakis gallinarum*, found in the ceca, or blind pouches, of a large percentage of chickens. This, together with the fact that chickens are not, as a rule, highly susceptible to histomoniatis, explains the frequent transmission of the disease from apparently healthy chickens to turkeys.

The parasites are capable of living for long periods in the cecal worm and its eggs. Van Es and Olney (1934) found that the infection remained on vacant yards from the middle of November until the middle of June during each of 5 years when turkeys were reared in the yards from June to November. For a more detailed discussion of the parasite and a description of the organism, reference is made to the works of Tyzzer (1920), Delaplane (1932), DeVolt and Davis (1936), Wehrich (1943), Connell (1950), and the chapter on protozoa. Recent reports which have aided in a better understanding of the etiology and pathogenesis of this disease include McGuire and Cavette (1952), DeLappe (1953,a,b,c,d), Harrison *et al.* (1954), Lund (1955, 1956, 1958a), Lund and Burtner (1957), Malewitz *et al.* (1958), and Goedbloed and Bool (1962). Lund (1963) described a new species of a histomonad, *H. wenrichi*, which inhabits the lumen of the cecum but does not apparently invade the tissues or produce the typical disease. For a more complete discussion of this species see Chapter 37. Figure 41 40 from Lund (1958b) illustrates how the cecal worm plays an important role in the transmission of this disease.

New evidence has been presented by Lund *et al.* (1963) that earthworms are true hosts of Heterakis and are thus of importance in transmitting blackhead. Because of this new discovery, the life cycle depicted in Figure 41.40 will need to be revised to include the earthworm.

Signs. "Blackhead," the common name, is a misnomer. Drowsiness, weakness, drooping wings and tail, a lowered head, ruffled feathers, and constant sulfur-colored diarrhea are characteristic symptoms. As a rule, adult birds are sick for several days and become emaciated before dying. Young poults may have an acute type of the disease and die soon after symptoms are noted. Although turkeys of all ages are susceptible, the heaviest losses occur during the first 12 weeks of life.

The mortality is high, often approaching 100 per cent, and averages about 50 per cent unless kept under control. Once the disease attacks a flock, occasional birds are likely to die between the intermittent periods of heavier losses, especially if the flock is not moved frequently to clean grounds. The period of incubation after contact with infection is 15 to 21 days.

Necropsy findings. The liver and the ceca are the principal organs showing marked changes. The severity of these changes varies with individuals. The cecal lesions are apparently the primary ones, and one or both ceca may be affected (Fig. 41.41). The lesions consist of marked inflammatory changes and ulcerations, sometimes involving most of the organ. A single ulcer may involve the serosa and pierce the wall. The mucous membrane often becomes necrotic, much thickened, and covered with a characteristic foul-smelling, yellowish-green, semi-caseous exudate; or a dry, hard, cheesy core may fill the cecum.

The affected liver (Fig. 41.42) presents a characteristic appearance, with areas of necrotic and degenerated tissues on the surface. These are more or less circular, have a yellowish to yellowish-green appearance, and in contrast to tumors and tubercles (tuberculosis), are somewhat depressed below the liver surface. They extend deeply into the tissue and are more or less confluent with the healthy tissue. In older birds the individual lesions are often merged. Evidence of healing is seen in the large amount of scar tissue in older birds.

FIG. 41.40 — Histomoniasis. Chart showing cycle of infection and role played by the cecal worm in the transmission of the disease. (Lund, 1958b.)

FIG. 41 41 — Ceca of a turkey affected with blackhead. Note the swollen condition of one cecum and the discolored diseased areas near the middle and at the tip. The other shows a single lesion near its middle portion. (Oroybill, Univ. of Calif.)

Occasionally, peritonitis and involvement of the other organs may be observed

Differential diagnosis. Histomoniasis must be differentiated from other diseases involving the liver and cecum. Chief among these are tuberculosis, tumors, and mycotic diseases. Demonstration of the causative agent by microscopic examination and culturing is necessary for final diagnosis.

Treatment. No drug or combination of drugs has been found entirely satisfactory for stopping losses once the disease has obtained a foothold in a flock. Nicotine products and phenothiazine have gained their reputation because of claimed successes in removal of cecal worms, thus aiding prevention of the disease. Neither agent has been proven effective against Histomonas meleagridis.

A large number of drugs have been screened for their possible effectiveness against this disease but only a few have shown promise. A review of the literature on the current status of the effectiveness of drugs for control of the disease is given by Horton-Smith and Long (1956), Jerstad (1957), Wehr et al. (1958), and McGuire et al. (1964). Among the drugs listed by Wehr et al. as having some therapeutic value are 4-nitro-benzenearsonic acid, 1-ethyl-3-(5-nitro-2-thiazolyl) urea, furazolidone, and 2-acetylamino-5-nitrothiazole. Welter and Clark (1961) reported successful treatment with p-ureido-benzenearsonic acid and Lindquist (1962) with an antibiotic paromomycin sulfate. McGuire et al. (1964) and McGregor et al. (1964) review the literature on the use of dimetridazole (1,2-dimethyl-5-nitroimidazole) and report additional trials on its use against the disease.

Inasmuch as rapid strides are being made on research in therapeutics, the reader is urged to review recent literature to determine if more effective drugs have been developed. Recommendations of manufacturers should be carefully observed when administering these drugs.

It should be emphasized that none of the drugs so far developed is highly effective once the disease has obtained a foothold in a flock. Furthermore, once treatment is discontinued, the disease is likely to reappear since little or no immunity is developed in infected birds and there is no cumulative action of the drugs tested. These facts indicate the need to realize treatment of any kind is only an adjunct to the prevention program outlined below.

Prevention. This is a "filth-borne" disease dependent on carriers, including not only chickens and turkeys but other birds

FIG. 41.42 — liver of turkey affected with blackhead. (Graybill, Univ. of Calif.)

as well. For a discussion of carriers other than chickens and turkeys the reader is referred to the chapter on protozoa. These carriers eliminate the causal organism in the feces, alone or within the cecal worm and its eggs. When the organism is ingested by susceptible stock, infection results. Both Horton-Smith and Long (1955) and Lund (1956) have shown that *Histomonas meleagridis* usually cannot live to establish an infection unless it is protected by some resistant body such as the egg of the cecal worm. The resistance of *H. meleagridis* when protected by cecal worms or their eggs was demonstrated by Farr (1956, 1961). She was able to recover both *H. meleagridis* and Heterakis larvae from turkeys fed droppings which had remained on soil for over 4 years. This also demonstrates the marked resistance of Heterakis eggs. This fact emphasizes the importance of the cecal worm in perpetuation and transmission of the disease. In turn, it emphasizes the importance of elimination of the cecal worm as a preventive measure in controlling the disease. The discovery by Lund *et al.* (1963) that both Heterakis and Histomonas may

be carried by earthworms and that blackhead may be transmitted by them complicates the prevention and control program.

Because there is no practicable method of identifying carriers, all chickens and turkeys must be under suspicion. Examples of methods of mechanical transmission of the disease are feed sacks and grains (corn, oats, wheat, etc.) that become contaminated with feces from chickens or turkeys harboring both the cecal worm and the *H. meleagridis*. Frank (1953) succeeded in transmitting the disease to turkeys by feeding grasshoppers that had been previously infected with cecal worm eggs.

Billings (1928) suggests a four-yard rearing system consisting of dividing 1 acre into four yards. These are divided as suggested in Figure 41.43. Three hundred poults can be raised in such a unit. The poults are reared for a month in each of the other yards in succession. They are moved each month until marketed. The acre of ground can be fenced into the ¼-acre sections, or the fence may be a temporary one, set up around a different section each month. Regardless of the system used in rearing
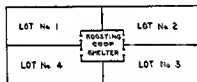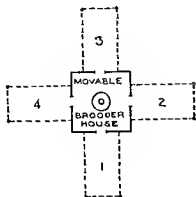
FIG. 41.43 — Two systems for rotation of runs suggested by Billings for prevention of blackhead. (Hinshaw, Univ. of Calif.)

turkeys, the following precautions must also be observed:

1. Keep the turkeys entirely separated from the chickens or chicken yards. Drain age from chicken yards to turkey yards is a common source of the disease.

2. Do not rear turkeys on ground that has been fertilized with chicken or turkey manure.

3. Do not rear turkeys in yards where losses have occurred until at least 2 years after the removal of the last diseased bird. (See Farr, 1961.)

4. Do not introduce new stock without quarantining it for 3 weeks before adding it to the flock.

5. Feed an adequate ration, with plenty of fresh, clean water.

According to Lund (1958b) no drug is yet known which will attack the cecal worm before it can transmit histomoniasis. Phenothiazine will, however, eliminate over 90 per cent of the cecal worms and thus reduce the danger of transmission. It will not affect the cecal worm eggs already in the soil, so it is only after continuous treatment over a period of 2 to 3 years that complete elimination can be expected. Phenothiazine has no effect on *H. meleagridis*. (See Wehr *et al.*, 1958.)

Such remedial measures as just described are not recommended for general use where blackhead can be readily controlled by methods not involving drugs. Nor are these remedies recommended as general procedures if blackhead has not been proven a problem.

REFERENCES

Billings, W. A. 1928. Talking turkey. Minn. Agr. Ext. Div., Spec. Bul. 124.

Connell, R. 1950. Enterohepatitis (blackhead) in turkeys. VI. Abnormalities possibly caused by a stage of *Histomonas meleagridis* occurring in second stage larvae of blackhead transmitting *Heterakis gallinae*. Canad. Jour. Comp. Med. 14:351.

Cuttice, C. 1907. The rearing and management of turkeys with special reference to the blackhead disease. R.I. Agr. Exper. Sta., Bul. 123.

Cushman, S. 1893. Experiments with turkeys. R.I. Agr. Exper. Sta., Rept. for 1893, p. 284.

Delaplane, J. P. 1932. Etiological studies of blackhead (enterohepatitis) in turkeys. R.I. Agr. Exper. Sta., Bul. 233.

DeLappe, I. P. 1953a. Studies on *Histomonas meleagridis*. I. Use of antibiotics to facilitate *in vitro* isolation. Exper. Parasit. 2:79.

——— 1953b. Studies on *Histomonas meleagridis*. II. Influence of age of original inoculum and pH on growth in various media. Exper. Parasit. 2:117.

——— 1953c. Studies on *Histomonas meleagridis*. III. The influence of anaerobic versus aerobic environments on the growth of the organism *in vitro*. Exper. Parasit. 2:209.

——— 1953d. Studies on *Histomonas meleagridis*. IV. A continuous automatic potentiometric method of measuring the E_h of protozoan cultures. Exper. Parasit. 2:280.

DeVolt, H. M., and Davis, C. R. 1936. Blackhead (infectious enterohepatitis) in turkeys, with notes on other internal protozoa. Md. Agr. Exper. Sta., Bul. 392:493.

Farr, M. M. 1956. Survival of the protozoan parasite *Histomonas meleagridis* in feces of infected birds. Cornell Vet. 46:178.

——— 1961. Further observations on survival of the Protozoan parasite, *Histomonas meleagridis* and eggs of poultry nematodes in feces of infected birds. Cornell Vet. 51:3.

Frank, J. F.: 1953. A note on the experimental transmission of enterohepatitis of turkeys by arthropods. Canad. Jour. Comp. Med. 17:230

Goodblood, E., and Bool, R. H.: 1962. The protozoan etiology of blackhead. Avian Dis. 6:302.

Harrison, A. P., Hansen, P. A., DeVolt, H. M., Holst, A. P., and Tromba, F. G.: 1954. Studies on the pathogenesis of infectious enterohepatitis (blackhead) of turkeys. Poultry Sci. 33 84.

Higgins, L. F.: 1915. Entero-hepatitis or black-head in turkeys. Canad. Dept. Agr., Health Anim. Branch, Bull. 17.

Horton-Smith, C., and Long, P. L.: 1955. The infection of chickens (Gallus gallus) with suspensions of blackhead organism Histomonas meleagridis. Vet. Record 67:378

——— and Long, P. L.: 1956. Furazolidone in the control of histomoniasis (blackhead) in turkeys. Jour. Comp. Path. Therap. 66.22.

Jerstad, A. C.: 1957. Furazolidone for infectious enterohepatitis (blackhead) of turkeys. Am. Jour. Vet. Res. 18:174.

Lindquist, W. D.: 1962. Some effects of paromomycin sulfate on histomoniasis in turkeys Am. Jour. Vet. Res. 23:1053.

Lund, E. E.: 1955. The progress of histomoniasis (blackhead) in turkeys as related to the size of the infective dose. Poultry Sci. 34:127.

———: 1956. Oral transmission of Histomonas in turkeys Poultry Sci. 35:900.

———: 1958a. Growth and development of Heterakis gallinae in turkeys and chickens infected with Histomonas meleagridis. Jour. Parasit. 44:297.

———: 1958b. War on blackhead still to be won. Turkey World 33(May) 12.

———: 1963. Histomonas wenrichi n. sp. (Mastigophora: Mastigamoebidae), a nonpathogenic parasite of gallinaceous birds. Jour. Protozool. 10:401.

——— and Burtner, R. H., Jr.: 1957. Infectivity of Heterakis gallinae eggs with Histomonas meleagridis. Exper. Parasit. 6:189.

——— Wehr, E. E., and Ellis, D. S.: 1963. Role of earthworms in transmission of Heterakis and Histomonas to turkeys and chickens. Jour. Parasit. 49 (Suppl.):50.

McGregor, J. K., Ferguson, A. E., Connell, M. C., and Morrison, W. D.: 1964. The relative activity of various drugs against experimental histomoniasis in turkeys. Poultry Sci. 43:1026.

McGuire, W. C., and Cavett, J. W: 1952 Blood studies on histomoniasis in turkeys. Poultry Sci. 31:810.

——— Moeller, M. W., and Morehouse, N. F.: 1964. The effect of dimetridazole on growth and the prevention of histomoniasis in poultry. Poultry Sci. 43:864.

Malewitz, T. D., Runnells, R. A., and Calhoun, M. L.: 1958. The pathology of experimentally produced histomoniasis in turkeys. Am. Jour. Vet. Res. 19.181.

Moore, V. A.: 1896. The direct transmission of infectious entero-hepatitis in turkeys. Bur. Anim. Ind., U.S.D.A., Circ. 5:1.

Smith, T.: 1895. An infectious disease among turkeys caused by protozoa (infectious enterohepatitis). Bur. Anim. Ind., U.S.D.A., Bul 8.7.

——— and Graybull, H. W. 1920. Blackhead in chickens and its experimental production by feeding embryonated eggs of Heterakis papillosa. Jour. Exper. Med. 32:143.

Tyzzer, E. E.: 1920. The flagellate character and reclassification of the parasite producing "blackhead" in turkeys—Histomonas (Gen. Nov.) meleagridis (Smith). Jour. Parasit. 6·124.

Van Es, L., and Olney, J. F.: 1934. Diseases of poultry—their nature and control. Nebr. Agr. Exper. Sta., Bul. 290.

Wehr, E. E., Farr, M. M., and McLoughlin, D. K.: 1958. Chemotherapy of blackhead in poultry. Jour. Am. Vet. Med. Assn. 132:439.

Welter, C. J., and Clark, D. T.: 1961. The efficacy of p-ureidobenzenearsonic acid as a preventative of histomoniasis in turkey poults. Poultry Sci. 40:144.

Wenrich, D. H.: 1943. Observations on the morphology of Histomonas (Protozoa, Mastigophora) from pheasants and chickens. Jour. Morph. 72.279

COCCIDIOSIS

Losses in young turkeys are often mistakenly ascribed to coccidiosis when some other disease is responsible. Most of the cases of bloody diarrhea in turkeys which are attributed to coccidia are not caused by these parasites.

Nine species of turkey coccidia have been described: Eimeria meleagridis, E. meleagrimitis, E. dispersa, E. gallopavonis, E. adenoeides, E. innocua, E. subrotunda, Isospora haisini, and Cryptosporidium meleagridis. These last two species described respectively by Svanbaev (1955) from USSR, and by Slavin (1955) from Scotland have not been reported from the United States.

Signs. The only two species which are highly pathogenic for poults are E. meleagrimitis and E. adenoeides (Hawkins, 1952; Moore and Brown, 1951, 1952). Hawkins, who first described E. gallopavonis, presented limited information to indicate that this species is also pathogenic.

This observation has been confirmed by Farr et al. (1961) and Wehr et al. who found this species highly pathogenic for 3- to 6-week-old poults and less so for 11-week-old poults. The other species can be considered nonpathogenic under normal environments. *E. meleagridis* may produce mild symptoms and slight losses under poor management (Hawkins, 1952; Moore et al., 1954).

The common clinical signs seen in outbreaks include listlessness, huddling into groups, constant cheeping, and lack of appetite beginning about the fourth day following infection and lasting for 4 to 5 days. Feed consumption falls on the fourth or fifth day but is resumed within a week unless the outbreak is especially acute. Rapid loss in weight occurs during this period. The peak of mortality is reached from the fifth to seventh day.

Hemorrhagic diarrhea as observed in *E. tenella* infections in chickens is not seen. Hawkins (1952) found that in poults artificially infected with *E. meleagrimitis* the droppings were scanty and slightly fluid with occasional blood flecks. At the peak of the infection (5 to 6 days), some of the fecal matter appeared in cylindrical forms 1 to 2 cm. in length and 3 to 6 mm. in diameter with sharply cut ends. Moore and Brown (1951) found the feces in severe *E. adenoeides* infections to be more fluid than normal, occasionally blood tinged, and containing mucous casts 1 to 2 inches long. These abnormal droppings appeared several hours previous to elimination of oocysts and persisted for about 4 days.

Age resistance has been noted for both the pathogenic species, but immunity following infection is only relative. Both species produce more acute symptoms in poults under 5 weeks of age with smaller dosages of oocysts than in older poults. In older poults, loss in weight over the short period of infection is the only noticeable symptom.

Necropsy findings. Hawkins (1952) summarizes the gross pathology of *E. meleagrimitis* infection as follows: Four days after infection, the duodenum and jejunum con-

tain an excessive amount of fluid. The jejunum is slightly thickened, dilated, and small areas of congestion are noted. The posterior part of the jejunum and the ileum may contain a greenish mucus. By the end of 5 to 6 days, the lumen of the small intestine is devoid of food, and the walls become covered with a white mucous deposit containing myriads of coccidia. If blood is present in the fluid, it is only in amounts sufficient to slightly tinge the liquid. Small flecks of clots may be noted. Mucous strands in the upper intestine are numerous. By the end of a week, irregular areas of congestion may be noted in the duodenum and jejunum. Greenish mucoid casts may be found in the posterior jejunum and in the ileum.

These are also essentially the findings of Moore and Brown (1951) for *E. adenoeides* infection. Thus, catarrhal enteritis, marked accumulation of fluid, which may be slightly blood tinged, and the presence of mucoid casts, which may be greenish, are the significant lesions. The site of the lesions may vary because of different locations of the sites of infection. The site of *E. meleagrimitis* infection is primarily in the jejunum, while the primary site for *E. adenoeides* is the lower ileum, rectum, and ceca. In severe infections with *E. gallopavonis* the mucosa of the lower small intestine and the proximal portions of the ceca show marked inflammatory changes (Farr et al., 1961; Wehr et al., 1962).

For greater details, histopathology, and differential characteristics between these and the other species the reader is referred to Chapter 37. Final diagnosis will depend on demonstration and identification of the coccidial species involved. The finding of coccidial oocysts alone is not evidence enough to warrant a diagnosis of coccidiosis as the cause of losses in a flock.

Prevention. In artificial brooding, preventive measures are more practicable than when turkeys are brooded naturally; but two important avenues of infection exist — namely, the feed and the attendant. Indirectly, the attendant is a carrier of coccidia by way of feed, especially if he shovels it from one pile to another on

floor, since he cannot avoid walking on feed mixed in this manner. Visitors are also potential mechanical carriers.

A rigorous sanitation program will aid in keeping coccidial infection reduced to a minimum and in turn aid in building up an immunity to the disease. Coccidial oocysts must have moisture in order to form spores, without which they cannot produce disease. Keeping thoroughly dry all areas to which poults have access will do much, therefore, to prevent acute outbreaks. Frequent changing of litter, the use of wire-screened platforms for water and feed containers, and ample floor space are aids in keeping the floors of the brooder houses dry.

Control and treatment. There are two objects for use of drugs. One is to give the drug at a low daily level in the feed during the susceptible age in order to prevent acute outbreaks and to aid in establishing immunity by keeping the number of oocysts reduced to a level below that needed to produce the acute disease. The second is to administer the drug early in an acute outbreak to prevent undue losses. In either case good management procedures are a necessary adjunct to successful control.

A number of drugs have been proven of value for control of coccidiosis in chickens. The same ones are effective in control of the disease in turkeys, so the reader is referred to the section on coccidiosis in Chapter 37 for details. There are only a few references to specific treatment of the disease in turkeys. Moore (1949) found that sulfaquinoxaline, sulfaguanidine, sulfamethazine, and sulfamerazine were of equal value for the control of the disease in turkeys. The percentage amount used in the feed for each drug was as follows: sulfaquinoxaline, 0.03 per cent; sulfaguanidine, 1.0 per cent, sulfamethazine, 0.5 per cent; and sulfamerazine, 0.5 per cent Morehouse (1949) tested six sulfonamides, including the ones referred to by Moore, and found five of them effective against coccidiosis of turkeys. Boyer and Brown (1953) review the literature on treatment of turkeys. Additional references to specific treatment of turkeys include Cuckler et al (1956) on the use of nicarbazin; Cuckler et al (1961) on the efficacy of amprolium; Ball and Warren (1963) on the use of the sulfaquinoxaline and amprolium; and Hymas and Stevenson (1962) on Zoalene (3,5-dinitro-0-toluamide) for both chickens and turkeys

REFERENCES

Ball, S. J., and Warren, E. W.: 1963 The effect of sulphaquinoxaline and amprolium against *Eimeria adenoeides* and *E. meleagridis* in turkeys. Res. Vet. Sci 4 39.

Boyer, A. C., and Brown, J. A.: 1953. The comparative coccidiostatic activity of some drugs against turkey coccidia. Proc. Book, 90th Ann. Meet Am. Vet. Med. Assn., p. 328.

Cuckler, A. C, Cobb, W. R., McManus, E. C., and Ott, W. H : 1961. Amprolium. 6. Efficacy for turkey coccidiosis. Poultry Sci. 40:1392.

———, Malanga, C. M., and Ott, W. H.: 1956. The antiparasitic activity of nicarbazin. Poultry Sci. 35:98.

Farr, M. M., Wehr, E. E., and Shalkop, W. T.: 1961 Pathogenicity of *Eimeria gallopavonis* Va. Jour. Sci. 12:150.

Hawkins, P. A.: 1952. Coccidiosis in turkeys. Mich. Agr. Exper. Sta . Tech. Bul. 226.

Hymas, T. A. and Stevenson, G. T.: 1962. A report on the efficacy of Zoalene 3,5-dinitro o-toluamide) as a control agent for coccidiosis in chickens and turkeys when included in feeds. Proc. Twelfth World's Poultry Cong., Sydney, Australia. P. 315.

Moore, E N.: 1949. Sulfaquinoxaline as a treatment for coccidiosis in turkeys. Cornell Vet 39 223

——— and Brown, J. A.: 1951. A new coccidium pathogenic for turkeys, *Eimeria adenoeides* n. sp. (Protozoa: Eimeriidae). Cornell Vet. 41:124.

———, and Brown, J. A.: 1952. A new coccidium of turkeys, *Eimeria innocua* n. sp. (Protozoa: Eimeriidae). Cornell Vet. 42:395.

———, Brown, J. A., and Carter, R. D.: 1954. A new coccidium of turkeys, *Eimeria subrotunda* n sp. (Protozoa: Eimeriidae). Poultry Sci. 33-925.

Slavin, D.: 1955. *Cryptosporidium meleagridis* n. sp. Jour. Comp. Path. and Therap. 65:262. (Quoted by Lund and Farr, Ch. 36)

Svanbaev, S. K.: 1955. (A new species of coccidia in turkeys) Trudy Inst. Zool Akad Mank. Kazak. USSR 3:161. (Quoted by Lund and Farr, Ch. 36)

Wehr, E. E., Farr, M. M., and Shalkop, W. T.: 1962. Studies on pathogenicity of *Eimeria gallopavonis* to turkeys. Jour. Protozool. 9:8.

HEXAMITIASIS

Hexamitiasis is a disease of young poults, causing its greatest mortality in those under 10 weeks of age. It was thought for many years that the disease was caused by trichomonads, but Hinshaw *et al.* (1938a, b) reported that a species of Hexamita and not a Trichomonas was responsible for the disease. They found that neither *T. eberthi* nor *T. gallinarum* (commonly found in the ceca of turkeys and chickens) was capable of producing a similar disease. The causal agent has been named *Hexamita meleagridis* by McNeil *et al* (1941). They describe the parasite as follows:

"This organism (minus flagella) varies in length from 6 to 12μ (average 9μ) and in width from 2 to 5μ (average 3μ). The nuclear membrane is distinct, and the karyosomes are round and fairly large (two-thirds diameter of the nucleus). Anterior to the nuclei are 2 large blepharoplasts (or groups of blepharoplasts) from which arise the 4 anterior and 2 anterolateral flagella. The flagella are all of about the same length, measured from the point of emergence from the body. The 4 anterior flagella are usually curved back along the body. Just posterior to these 2 large blepharoplasts are 2 others

from which arise the 2 caudal flagella. These flagella pass posteriorly in a granular line of cytoplasm to their pockets of emergence near the posterior end of the body" (Fig. 41.44A and B).

Slavin and Wilson (1953, 1960) have done an exhaustive morphological study of Hexamita from turkeys in Scotland. Their studies indicate that *H. meleagridis* does not divide by simple binary fission, and that a reproductive cycle can be demonstrated in the reticuloendothelial cells and tissue interspaces of the bowel wall.

The disease is prevalent in most sections of the United States. Published reports in addition to those from California include one from Connecticut by Jungherr and Gifford (1944) and one from Indiana by Doyle *et al.* (1947). Campbell (1945) reported the presence of Hexamita in turkeys, infected also with Cochlosoma, in an outbreak in Scotland. He considered that Cochlosoma was the primary cause of losses in this outbreak. The reports of Slavin and Wilson (1953) and Wilson and Slavin (1955) further establish the existence of the disease in Scotland. Vance and Bigland (1956) have reported it from Canada.

Signs. In the early stages of acute outbreaks the poults are nervous and require
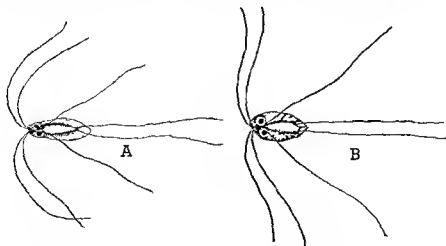
FIG. 41.44 — (A, B) *Hexamita meleagridis* from the intestine of the turkey, showing individual variation in size and shape. X1,875. (McNeil, Hinshaw, and Kofoid, Am. Jour. Hyg.)

more heat than normally; the body temperature is normal or subnormal; the gait is stilted; and the feathers are ruffled and unkempt. There is foamy watery diarrhea, but the cecal droppings do not appear changed.

In most cases the poults continue to eat and may even appear to consume more feed due to a nervousness that is always evident. This nervousness is also manifested by the continual chirping of the birds, especially in the early stages. Due to improper digestion and assimilation of feed, the poults lose weight rapidly. Many of the survivors continue to be underweight for weeks. In the later stages of the disease the poults become listless, sit under the hover, and go into a coma. Finally, they struggle, flap their wings, and die.

Subacute attacks of the disease may occur in young poults early in the season before the infection reaches the epidemic stage. Milder outbreaks may occur in poults that reach a resistant age. In such outbreaks, listlessness and loss of weight are the most prominent symptoms. Loss of appetite will depend on the severity of the disease. Large numbers of stunted individuals result from this form.

Course and mortality. In experimentally produced outbreaks symptoms appear 4 to 7 days after ingestion of the parasites. The period of incubation varies with the amount of inoculum and the age of the individual. If temperatures are taken daily following infection, a drop will often be noted a day before visible symptoms are seen. Likewise, daily weight records will usually show a decline in the daily gain before symptoms appear.

Mortality may start within a day after symptoms appear. In acute outbreaks the course of the disease is typical of an acute infectious disease, with the peak of mortality occurring in 7 to 10 days following the appearance of symptoms. In most instances straggling losses occur for as long as 3 weeks, and in a few flocks, a second peak of mortality has been observed.

In outbreaks complicated with other infections, such as salmonellosis, or by faulty management, the course may be varied and the mortality increased. Heavy losses seldom occur in poults over 10 weeks of age unless there has been some lowering of resistance due to another infection or due to environmental factors. It is always desirable to eliminate the possibility of such complications whenever losses associated with the presence of *Hexamita meleagridis* are encountered in older turkeys.

Environmental and husbandry factors, as well as age, greatly influence the mortality. It may vary from a few poults to the entire flock. Under experimental conditions, using normal poults, we have not been able to produce heavy mortality in poults over 8 weeks of age.

Necropsy findings. At necropsy the birds are in poor condition; the feathers lack luster; the skin is dry; the flesh of the breast is dehydrated and reddened. In young poults suffering from an acute outbreak, the crop usually contains some food; in poults that linger longer before death, it is usually empty.

The principal pathological change occurs in the duodenum, jejunum, and ileum, where there is catarrhal inflammation with marked lack of tone. The intestinal contents may vary from a thick mucous type to a thin watery, foamy type. The latter is most characteristic. Localized bulbous areas filled with watery contents are also characteristic. The mucous membrane of these areas is often congested.

The contents of the ceca may be more fluid than normal, but seldom changed otherwise. The only change noted in the ceca is a congestion of the cecal tonsils.

Diagnosis. Diagnosis must be based on finding Hexamita in the upper intestines. The examination of cecal or rectal contents is not recommended as a diagnostic procedure because of the necessity of having to differentiate other flagellates from *Hexamita meleagridis* (Fig. 41.11). If smears are made from the duodenum or jejunum, and diluted with physiological saline, Hexamita, if present, will usually be found free from other flagellates.

Cochlosoma, a flagellate first reported in ducks by Kimura (1934), has been found by McNeil and Hinshaw (1942) associated with *Hexamita meleagridis* in a few outbreaks. Cochlosoma can be readily distinguished from Hexamita by its characteristic rolling movement and its cochleal shape. It is necessary, for best results, to examine poults that have recently died, but the parasites have been found in refrigerated specimens 48 hours after death. It may be necessary to warm the slide at 35° to 40° C. for a few minutes if the smears are taken from such specimens

The parasites may also be found in the bursa of Fabricius; in the carrier stage they localize in this organ as well as in the cecal tonsils. When the acute disease subsides, Hexamita can seldom be found in the upper intestines.

Transmissible enteritis (avian monocytosis, bluecomb) of turkeys, which is now recognized as being caused by a filterable agent (Sieburth and Johnson, 1957; and Tumlin *et al.*, 1957), must be differentiated from hexamitiasis because of many characteristics in common. McNeil (1958) suggests that in many field cases transmissible enteritis may occur simultaneously with hexamitiasis with a resultant increased severity in the total outbreak. She also postulates that virus particles of the disease may become attached to Hexamita and become transmitted in this manner. If this is true, then other viruses like that causing viral hepatitis could possibly be transmitted in the same manner. The reader is referred to the chapter on Avian Monocytosis and to the section on transmissible enteritis in this chapter for a more complete discussion.

Transmission. McNeil *et al.* (1939) found *Hexamita meleagridis* in California valley quail, Gambel's quail, and in chukar partridges. Hinshaw and McNeil (1941), in a survey of possible carriers, found Hexamita in 16.5 per cent of 79 live adult turkeys by rectal examination and in 32.4 per cent of 74 turkeys by examination of scrapings from the cecal tonsil at necropsy. All these birds were survivors

of acute outbreaks. They also examined 11 species of game and wild birds other than quail and chukars killed on or near infected ranches. None harbored Hexamita. Chickens, ducks, pigeons, and guinea fowl were negative for *Hexamita meleagridis*, but pigeons harbored *Hexamita columbae* (McNeil and Hinshaw, 1941a) Ducks and chickens were artificially infected with *Hexamita meleagridis*, but symptoms of the disease were not produced Kimura (1934), in a paper on Cochlosoma in ducks, mentions the occurrence of Hexamita in the ceca and large intestine of domesticated ducks in California. McNeil and Hinshaw (1941b) found the parasite in chickens artificially infected 22 weeks after inoculation. Hexamita has also been reported in peafowl. (California State Department of Agriculture, 1941) and in pheasants by Hinshaw and McNeil (1942) and Stover (1943). Thus, surviving turkeys, quail, chukars, pheasants, peafowl, chickens, and ducks must be considered potential carriers of *Hexamita meleagridis*.

No insect transmitter has been found. It has consistently been possible to keep noninfected poults free from the disease when reared in proximity to infected brooders even though flies were abundant.

Epizootiology. Management and environment play important roles in the transmission of this disease. The adult turkey that has survived an outbreak is the most important factor in perpetuation of the disease on a ranch. Chukar partridges, quail, pheasants, peafowl, and ducks may also play a part in starting an outbreak. Even though chickens have not been found infected under natural conditions, the fact that they may be artificially infected means that the parasite may sometimes be adapted to them, and they must be considered potential carriers. Outbreaks of hexamitiasis have been definitely traced to infected quail and pheasants ranging with turkeys.

In the field the acute disease is usually seen in the later hatches and often after the breeders have been marketed. Thus

adult transmission is often difficult for the owner to understand, because his earlier groups did not show symptoms. The explanation, based on experimental and field studies, is that the early hatched poults act as a reservoir for increasing the dosage and probably the virulence of the parasites and serve as the intermediary transmitter from the breeders to the later hatched poults. Experimentally, it takes from three to five passages of parasites removed from turkeys in the carrier stage through young poults before the infection is increased to insure acute outbreaks.

Environmental and husbandry factors also play an important part in establishing the infection in epidemic proportions. In most instances, as the brooding season advances, the volume of work on the ranch increases, and the space available per bird is less, thus increasing the chances for spread of disease. The difficulties arising with the hot weather, and in some areas the late spring fogs, also contribute to the development of the disease in later broods.

Prevention, control, and treatment. The primary source of infection is the intestinal contents of carriers. The entire program of prevention must be built around the recognition of this fact. Finding a satisfactory method of preventing the transfer of droppings from carriers to young birds is the most efficient method of preventing disease. *No general recommendations as to the best procedure to follow can be given because every ranch requires a separate solution of the problem of eliminating the danger of having carriers on the ranch.*

Factors which will aid in solving the individual problems are:

1. Separate units and caretakers for the breeding flock and the young poults.
2. Separate equipment for each age group.
3. Intelligent use of wire platforms for feed and water.
4. Intelligent use of cement yards and wire pens.
5. Arrangement of feeding and watering equipment for easy access to the attendant

without entering the pen, to avoid contamination.

6. If the poults have undergone an outbreak of pullorum disease or paratyphoid infection, avoid changes in brooding until they are 12 to 16 weeks of age.
7. Sell all breeding birds 2 weeks before any poults are hatched.
8. Avoid ranges frequented by pheasants, quail, and chukars.

An accurate laboratory diagnosis is the first essential in the advent of a suspected outbreak. Live sick birds are necessary for the accurate diagnosis of hexamitiasis, although Hexamita may be found as long as 48 hours after death of the poult if decomposition has not advanced too far.

Complete isolation and quarantine of infected pens to prevent spread of the disease to normal poults is the most important factor in the control program. *Keeping the poults warm by increasing the heat in the brooder house and increased effort to keep them comfortable are essential.* Removal and destruction by burial or burning of all dead poults several times daily, and daily dry cleaning of the houses and yards are essential to prevent spread of the infection.

Drugs and combinations of drugs that have been tried experimentally and proved to have no therapeutic value include mercuric chloride (1:8,000 and 1:4,000 as a substitute for drinking water) (McNeil and Hinshaw, 1945); sodium bicarbonate (baking soda), copper sulfate, nicotine sulfate, iodine, sulfonamides, penicillin, and several arsenical preparations.

McNeil (1948) obtained promising results by the use of a mixture of 3 per cent by weight of dried whey in a 1:2,000 aqueous solution of copper sulfate. This mixture, given in place of all drinking water for several days beginning early in an outbreak, should only be used as an adjunct to the recommended sanitary and management program. The cause of action of this combination on the disease is not known, but it is believed to be associated with the effect of the lactose-copper combination on lower intestine metabo-

lism (McNeil et al., 1948). Dried whey containing from 50 to 70 per cent lactose is essential, and it must not be caramelized. The mixture must be kept fresh, stirred frequently, replaced often, and kept in clean containers. If poults fail to drink it readily after 3 to 4 days, it should be replaced with fresh water for a few days. The usual experience following its use is an increased appetite with marked increase in feed consumption.

Almquist and Johnson (1951) did a preliminary screening test on four antibiotics using 9-week-old poults in which the disease was artificially produced. Streptomycin was of no value, but encouraging results were obtained with Aureomycin (200 mg. per pound of mash), and Terramycin (25 mg. per poult), and penicillin-G (25 mg. per poult). The treatments were continued for 3 to 14 days. They also fed 2-amino-5-nitrothiazole (ANT) at the rate of 0.1 per cent in the mash for 14 days with good results.

Mangum et al. (1955) obtained favorable results with furazolidone when it was added to the ration at the rate of 50 mg. per pound of feed. Wilson and Slavin (1955) tried a number of drugs and found most of them of no value. Enheptin (Entamin in England), 2-amino-5-nitrothiazole, was never more than 50 per cent effective. Di-n-butyltin dilaurate (Tinostat) appeared to control mild outbreaks and lower death rates in severe field outbreaks, but had no effect when given to birds experimentally infected with a lethal dose of the parasite. They state that any drug to be effective would have to be one which could be given in the drinking water because infected birds cease to eat early in the course of the disease. Fogg (1957) found Nithiazide an effective treatment in field outbreaks when given in drinking water at the rate of 0.02 per cent. Again it is emphasized that drugs are not substitutes for preventive management.

REFERENCES

Almquist, H. J., and Johnson, C.: 1951. Antibiotics and hexamitiasis in turkeys. Proc. Soc. Exper. Biol. Med. 76:522.

California State Department of Agriculture: 1941. Hexamitiasis in peafowl. Ann. Rep. Petaluma Poultry Path. Lab., Calif. St. Dept. Agr., Monthly Bul. 30:435.

Campbell, J. G.: 1945. An infectious enteritis of young turkeys associated with Cochlosoma sp. Vet. Jour. 101:255.

Doyle, L. P., Cable, R. M., and Moses, H. E.: 1947. A destructive turkey disease. Jour. Am. Vet. Med. Assn. 111:57.

Fogg, D. E.: 1957. Nithiazide (Hepzide). Proc., Merck, Poultry Nutr. and Health Symp. St. Louis, Mo., Aug. 5, 1957, p. 16.

Hinshaw, W. R., and McNeil, E.: 1941. Carriers of Hexamita meleagridis. Am. Jour. Vet. Res. 2:453.

———, and McNeil, E.: 1942 Hexamita sp from the ring necked pheasant. Jour. Am. Vet. Med. Assn. 101:503.

———, McNeil, E., and Kofoid, C. A.: 1938a. The presence and distribution of Hexamita sp. in turkeys in California. Jour. Am. Vet. Med. Assn. 93:160.

———, McNeil, E., and Kofoid, C. A.: 1938b. The relationship of Hexamita sp. to an enteritis of turkey poults. Cornell Vet. 28:281.

Jungherr, E., and Gifford, R.: 1944. Three hitherto unreported turkey diseases in Connecticut; erysipelas, hexamitiasis, mycotic encephalomalacia. Cornell Vet. 34:214.

Kimura, G. G.: 1934. Cochlosoma rostratum sp. nov. an intestinal flagellate of domestic ducks. Trans. Am. Micr. Soc. 53:102.

———, 1938. Hexamitiasis of turkey poults. Calif. Agr. 2(11):15.

———, 1938. Hexamitiasis. Merck Agr. Memo. 3(Aug.-Sept., 1):5.

———, and Hinshaw, W. R.: 1941a. The occurrence of Hexamita (Octomitus) columbae in pigeons in California. Jour. Parasit. 27:185.

———, and Hinshaw, W. R.: 1941b. Experimental infection of chicks with Hexamita meleagridis Cornell Vet. 31:545.

———, and Hinshaw, W. R.: 1942. Cochlosoma rostratum from the turkey. Jour. Parasit. 28:549.

———, and Hinshaw, W. R.: 1945. Effect of mercuric chloride on turkeys and on Hexamita meleagridis. Poultry Sci. 24:156.

———, Hinshaw, W. R., and Kofoid, C. A.: 1941. *Hexamita meleagridis* sp. nov. from the turkey. Am. Jour. Hyg. 34:(Sec. C)71.

———, Kissling, R. E., and Hinshaw, W. R.: 1948 Host-parasite relationships in hexamitiasis of turkey poults. (Abstract.) Poultry Sci. 27.675

———, Platt, E. D., and Hinshaw, W. R.: 1939. *Hexamita sp* from quail and from chukar partridges. Cornell Vet. 29:330.

Mangrum, J. F., Ferguson, T. M., Couch, J. R., Wills, F. K., and Delaplane, J. P.: 1955. The effectiveness of furazolidone in the control of hexamitiasis in turkey poults. Poultry Sci 34:836.

Sieburth, J. McN., and Johnson, E. P.: 1957. Transmissible enteritis of turkeys (bluecomb disease). I. Preliminary studies. Poultry Sci. 36.256

Slavin, D., and Wilson, J. E.: 1953. *Hexamita meleagridis* Nature 172.1179.

——— and Wilson, J. E.: 1960. A fuller conception of the life cycle of *Hexamita meleagridis.* Poultry Sci. 39:1559.

Stover, D. E.: 1913. *Hexamita sp.* from the ring-necked pheasant transmissible to turkeys. Jour. Am. Vet. Med. Assn. 103-37.

Tumlin, J. T., Pomeroy, B. S., and Lindorfer, R. K.: 1957. Bluecomb disease of turkeys. IV. Demonstration of a filterable agent Jour Am. Vet. Med. Assn. 130 360.

Vance, H. N., and Bigland, C. H.: 1956. Hexamitiasis: A report of cases in Alberta turkey flocks, Canad. Jour. Comp. Med. and Vet. Sci 20:337

Wilson, J. E., and Slavin, D.: 1955 Hexamitiasis of turkeys Vet. Record 67.237.

LEUCOCYTOZOON INFECTIONS*

Smith (1895) discovered a protozoan parasite in the blood of turkeys, which later was named by Volkmar (1930) *Leucocytozoon smithi.* According to Wenyon (1926), Laveran and Lucet in 1905 found a similar blood parasite in turkeys in France. Volkmar in 1930 reported the parasite in turkeys from Minnesota and North Dakota, and Skidmore (1932) reported an outbreak of disease in turkeys from Nebraska caused by *Leucocytozoon smithi.* Skidmore presented evidence that black flies identified as *Simulium occidentale* (Townsend) were the transmitters of the parasite from turkey to turkey.

Johnson et al. (1938), Travis et al. (1939), and West and Starr (1910) have published the results of extensive studies on Leucocytozoon infections and possible carriers. Other published reports on the disease in North America include the following: California, by Hinshaw and McNeil (1943); Texas, by Banks (1943); and Manitoba, Canada, by Savage and Isa (1915).

For a complete description of the parasite and the disease, the reader is referred to Johnson et al. (1938), Johnson (1942), Bierer (1954), and Wehr (1962).

Signs. Poults under 12 weeks are most affected. Loss of appetite, droopiness, and a tendency to sit are common signs. Visible signs seldom last over 2 or 3 days, after which the birds either die or start to recover. When disturbed they move with difficulty, and in the later stages may fall over, gasp, go into coma, and die.

Recovered birds may suffer no serious after effects, but they may carry the parasite in the blood for months. Some individuals develop a chronic type of the disease. Male birds carrying large numbers of the organism in the blood rarely strut or pay any attention to the female.

Moist tracheal rales are common in chronic cases, and the affected individuals make repeated attempts to clear their throats when excited. Death may result from undue excitement or handling of such birds.

Necropsy findings. Slight inflammation of the duodenum is the only consistent gross lesion noted in young birds. Anemia and various degrees of emaciation may be noted, the flesh is flabby, and the musculature is of a brownish color.

In adult carriers no gross lesions are seen as a rule, but occasionally the liver is icteric, enlarged, and cirrhotic. Johnson and his associates believe that the respiratory symptoms are due to circulatory obstruction by large numbers of parasites, resulting in anemia of some of the vital organs. The pathology of the disease is described in detail by Newberne (1955).

Diagnosis. Microscopic examination of the tissues of diseased birds reveals large numbers of the parasites (Fig. 41.15).

* See also Chapter 37.

Dried blood smears stained according to Wright's or Giemsa's method make satisfactory specimens for examination. Finding one of the species of Simulium feeding on turkeys is further evidence that a Leucocytozoon is involved.

Transmission. Skidmore (1932), Johnson et al. (1938), Underhill (1939), and Bierer (1954) have shown that at least three species of Simulium, *Simulium occidentale*, *S. nigroparvum*, and *S. slossonae*, may transmit leucocytozoa by biting turkeys. These are very small stout-bodied, black flies which live along streams. (See also chapter on External Parasites.)

Prevention and control. No satisfactory treatment has been reported. Confinement-rearing in houses screened against simuliids until the poults are several weeks old is considered practical by Johnson (1939). It is necessary to make the houses for such method of rearing fly proof by the use of cheesecloth. Screen of 16 mesh to the inch failed to keep the flies from entering houses. According to Bierer (1954), the use of DDT or other insecticides for large area control of the fly transmitters has not been successful, although a few individual turkey growers have reported success by this preventive measure.

Complete segregation of breeding and brooding operations will do much to prevent transmission from adult carriers to poults. Selling of adult breeders, before the poults that are to be kept for replacements are hatched, is recommended.

Johnson et al (1938) failed to find the parasites in pheasants, ruffed grouse, crows, hawks, and buzzards, but did find them in wild turkeys. Travis et al. (1939) examined chickens, peafowls, guinea hens, and ducks on infected ranches and found them to be negative for leucocytozoa. They found wild turkeys infected in Missouri, Georgia, and Florida. Therefore, wild turkeys may be a source of infection, and their control must be considered in a prevention program. The same precautions recommended for hexamitiasis will also help in preventing this disease.

REFERENCES

Banks, W. C.: 1943. *Leucocytozoon smithi* infection and other diseases of turkey poults in central Texas. Jour. Am. Vet. Med. Assn. 102:467.

Bierer, B. W.: 1954. Buffalo gnats and leucocytozoon infections of poultry. Vet. Med. 49:107. Poultry Sci. 32:268.

Hinshaw, W. R., and McNeil, E.: 1943. *Leucocytozoon sp.* from turkeys in California. Poultry

Johnson, E. P.: 1939. A method of raising turkeys in confinement to prevent parasitic diseases. Va. Agr. Exper. Sta., Bul. 325.

———: 1942. Further observations on a blood protozoon of turkeys transmitted by *Simulium nigroparvum* (Twinn). Am. Jour. Vet. Res. 3:214.

Underhill, G. W., Cox, J. A., and Threlkeld, W. L.: 1938. A blood protozoon of turkeys transmitted by *Simulium nigroparvum* (Twinn). Am. Jour. Hyg. 27:649.

Newberne, J. W.: 1955. The pathology of leucocytozoon infection in turkeys with a note on its tissue stages. Am. Jour. Vet. Res. 16 593.

Savage, A., and Isa, J. M.: 1945. An outbreak of leucocytozoon disease in turkeys. Cornell Vet. 35:270.

FIG. 41.45 — Photomicrographs of stained turkey blood containing various stages of Leucocytozoon from turkeys. X750. Giemsa's stain.
(1) A microgametocyte with only one lateral bar present. Note light color of parasite.
(2) A microgametocyte with only one lateral bar present. Note dark color of parasite.
(3) A macrogametocyte with one bar on one side and two bars on opposite sides.
(4) A macrogametocyte shown as a, and the macrogametocyte at b, each with bilateral bars, are the most common forms found. At c may be seen a microgametocyte that has become round to form a macrogamete. Note that one bar is still attached ventrally.
(5) An early microgametocyte with distinct difference between density of central body, or what might be the parasite proper, and the surrounding cytoplasm is a typical granulocyte with the two lateral bars or possible host-cell nuclei. At the right and ventrally is well-marked distinction with eosinophilic rod granules. Note comparative size.
(6) The earliest macrogametocyte found. Here, again, there is well-marked distinction between central body and surrounding cytoplasm including the bilateral bars. (Johnson et al., Am. Jour. Hyg.)

Skidmore, L. V.: 1932. *Leucocytozoon smithi* infection in turkeys and its transmission by *Simulium occidentale* (Townsend) Zentralbl f Bakt 1 Orig 125:329.

Smith, T.: 1895. Infectious diseases among poultry. U.S.D.A., Bur. Anim Ind., Bull. 8:7.

Travis, B. V., Goodwin, M. H., Jr., and Gambrell, E 1939 Preliminary note on the occurrence of *Leucocytozoon smithi* Laveran and Lucet (1905) in turkeys in southeastern United States. Jour. Parasit. 25:278

Underhill, G. W.: 1939. Two simuliids found feeding on turkeys in Virginia. Jour. Econ. Entom. 32 765.

Volkmar, F.: 1930. Observations on *Leucocytozoon smithi*, with notes on leucocytozoa in other poultry. Jour. Parasit. 16 21.

Welh, E. E.: 1932. Studies on leucocytozoonosis of turkeys, with notes on schizogony, transmission, and control of *Leucocytozoon smithi.* Avian Dis 6:195.

Wenyon, C. M.: 1926. Protozoology. William Wood and Co., New York.

West, J. L., and Starr, L. E.: 1940. Further observations on a blood protozoan infection in turkeys. Vet. Med 35 619

TRICHOMONIASIS OF THE UPPER DIGESTIVE TRACT

History. Jungherr (1927) was the first to describe this clinical entity. At the time he suggested that a fungus was the probable cause. Three years later Volkmar (1930) reported that *Trichomonas diversa*, now designated as *Trichomonas gallinae*, is present in the crops of turkeys suffering from this disease. Hawn (1937) later showed this parasite to be the etiological agent. Stabler (1938a, b, 1954) has shown the similarity of this trichomonad to the one in pigeons, which he points out should be known as *T. gallinae* instead of *T. columbae*. He suggested that the species in turkeys be called by this name instead of *T. diversa.*

Levine *et al* (1941) reported evidence that the species found in turkeys is the same as that found in chickens and pigeons They were able to produce the typical disease in turkeys, chickens, bobwhite quail, canaries, and English sparrows with a species isolated from chickens.

Epizootiology. Most of the cases studied by Hinshaw have been in turkeys from 16 to 30 weeks of age reared on range land. The following description of one outbreak in California is typical of the environment in which most of the cases are found:

The turkeys involved in this outbreak had been reared on the home ranch under semiconfinement methods until the middle of September, when they were driven daily to a cut-over rice field about ½ mile from the ranch. Each day the birds were allowed to feed for 2 or 3 hours on the shattered rice left by the harvester. After this procedure had been continued for about a month, the flock was permanently moved to the rice field and allowed to range at will. They were fed a mash supplement that was left near the roosts located on a dry area in a cut-over barley field adjacent to the rice. Water was hauled from the home ranch, but the birds had access to the sluggish, algae-contaminated water in an irrigation ditch, which they had to cross in order to reach the rice from the roosting and mash-feeding areas. Seepage from the ditch had caused a large, muddy, stagnant pool to form near the edge of the rice field. The turkeys drank a great deal of this water and picked up the rice in the mud at the edge of the pool. Several similar pools were found in other parts of the field. The disease started within 10 days after the birds were permanently located on the rice field. Pigeons, abundant in the area, may have been the transmitting agent.

Signs. The signs are similar to those seen in many other diseases. Darkened heads with sunken sinuses and a generally haggard appearance are characteristic. The chest always has a depressed appearance, with the crop empty and drawn in towards the body. This typical attitude is seen in Figure 41.46. Lack of appetite, drooling from the mouth, roughened unkempt feathers, and a normal or slightly subnormal temperature are also observed. Diarrhea does not, as a rule, accompany the disease. A foul odor is always present. The course of the disease varies, but as a rule, it

FIG. 41.46 — Posture typical in trichomoniasis of the crop. Note especially the sunken appearance of the crop area. (Hinshaw, Univ. of Calif.)

is prolonged, and the birds become emaciated before death.

Necropsy findings. Chronic ulceration of the crop is the most common necropsy finding. The lower esophagus and, less often, the proventriculus and upper esophagus may be involved. The lower digestive tract and the other organs are, as a rule, normal. Aspergillosis of the lungs may be secondary to the necrotic ulceration of the upper digestive tract.

The lesions involve the glandular tissue and vary in size from a few to 15 mm. in diameter at the base (Figs. 41.47A and B, and 41.48). They taper to a point in concentric rings of piled-up necrotic tissue to as much as 5 mm. above the surface. They may extend into the tissue 3 or 4 mm. The surface protruding into the lumen of the organ is rough, irregular,

and surrounded at the base by a circular hemorrhagic ring. The lesions in the esophagus are usually smaller than those in the crop but are similar in shape and structure. When the proventriculus is involved, the esophageal portion is most affected. The lesions in the proventriculus are, as a rule, coalesced and may appear as a solid ring of necrotic material causing a marked thickening of the tissues and resulting in partial to complete occlusion of the lumen. Impactions of the lower esophagus have been noted in such cases.

Prevention and control. Since the disease is directly associated with insanitary surroundings, sanitation is of primary importance. Contact with pigeons should also be avoided.

The first requisite for control is sanitation. As soon as the disease is observed,
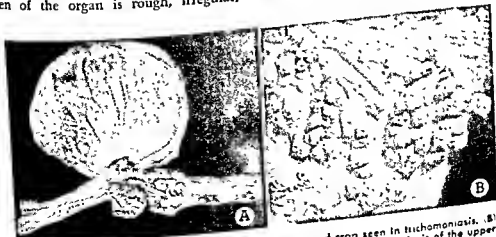
FIG. 41.47 — (A) Necrotic ulceration of the esophagus and crop seen in trichomoniasis. (B) Close-up of typical pyramidlike necrotic ulcers characteristic of trichomoniasis of the upper digestive tract. (Hinshaw, Univ. of Calif.)

FIG. 41.48 — Necrotic ulceration of the proventriculus often seen in trichomoniasis of this organ. (Hinshaw, Univ. of Calif.)

the flock should be moved to a dry, clean area and given plenty of pure, fresh water to drink. Sick birds should be kept separate and cared for by a person who has no contact with the healthy birds. Removing the causal agent and giving the birds good care is more essential than treatment with drugs.

Jacquette (1948) found copper sulfate of value in treating the comparable disease in pigeons. Other reports on use of drugs for the disease in pigeons are Stabler and Mellentin (1953), Samberg and Bornstein (1955), Stabler et al. (1958), and Bussiéras et al (1961). Stabler et al reported on the successful use of soluble Enheptin (2-amino-5-nitrothiazole) at the rate of 6.3 grams per gallon of water for pigeons. Bussiéras et al. reported on a drug which shows considerable promise for treatment of trichomoniasis in many hosts. It is metronidazole (1-beta-hydroxyethyl-2-methyl-5-nitroimidazole). It prevented mortality in pigeons when given orally at the rate of 60 mg/kg body weight over a 5-day period. One advantage over Enheptin is that it reportedly does not affect fertility of breeders. As far as is known the drugs mentioned have not been used against the disease in turkeys.

For a detailed discussion on T. gallinae as well as on other species the reader is referred to Chapter 37.

REFERENCES

Bussiéras, J., Dams, R., and Estéby, J. 1961. Prophylaxie de la trichomonose du pigeon par le Metronidazole Bul. Soc. Sci. Vét. Lyon 63:307. (Abst. 2245-Vet. Bul. 32,439)

Hawn, M C 1937. Trichomoniasis of turkeys. Jour. Infect. Dis. 61:184.

Jacquette, D S 1948 Copper sulfate as a treatment for subclinical trichomoniasis in pigeons. Am. Jour. Vet Res 9 206

Jungherr, E 1927. Two interesting turkey diseases. Jour. Am. Vet. Med. Assn, 71:636.

Levine, N. D., Boley, L. E., and Hester, H. R.: 1941. Experimental transmission of Trichomonas gallinae from the chicken to other birds. Am. Jour. Hyg. 33:(Sec. C) 23.

Samberg, Y., and Bornstein, S. 1955. In vitro action of various chemical agents on Trichomonas gallinae Poultry Sci 34:157.

Stabler, R. M.· 1958a. The similarity between the flagellate of turkey trichomoniasis and T. columbae in the pigeon. Jour. Am. Vet. Med. Assn. 93.33.

———— 1958b Trichomonas gallinae (Rivolta, 1878) the correct name for the flagellate in the mouth, crop, and liver of the pigeon. Jour. Parasit. 24:553.

———— 1954. Trichomonas gallinae. a review. Exper. Parasit. 3.368.

————, and Mellentin, R W. 1953. Effect of 2 amino-5 nitrothiazole (Enheptin) and other drugs on Trichomonas gallinae infection in the domestic pigeon. Jour. Parasit. 39.637.

————, Schmittner, S M, and Harmon, W. M.: 1958. Success of soluble 2-amion-5-nitrothiazole in the treatment of trichomonasis in the domestic pigeon. Poultry Sci. 37:352.

Volkmar, F.: 1930 Trichomonas diversa n sp and its association with a disease of turkeys Jour. Parasit. 17:85.

MISCELLANEOUS PROTOZOAN DISEASES

Protozoan infections that have been reported one or more times, and which may in the future prove to be significant causes of mortality, are briefly described below.

A *Cochlosoma*, first described by Kotlán (1923) from ducks in Europe, was named by him *C. anatis*. Kimura (1934) described a species from American ducks which he called *C. rostratum*. Travis (1938), in a synopsis of the genus, considers these species synonymous. McNeil and Hinshaw (1942) described a species in turkeys which was apparently the same species as described by Kotlán and Kimura. Campbell (1945) found *Cochlosoma* in turkey poults in Scotland. The true pathogenic significance of this flagellate is not known, although both Kotlán and Campbell suggest that it may be the cause of enteritis. Hinshaw has always found it associated with *Hexamita* or *Salmonella*.

Haemoproteus has been found by Morehouse (1915) in turkeys in Texas. Banks (1943) also suggested its presence in turkeys in Texas. This genus is usually carried by flies of the genus *Pseudolynchia*. Morehouse does not mention the insect vector for the species found in turkeys. Rivero (1947) has reported that the cone nosed bug, *Triatoma*, is able to transmit *Haemoproteus columbae*.

Herman (1941) described *Plasmodium durae* as the cause of bird malaria in a turkey in Kenya Colony, British East Africa.

McNeil and Hinshaw (1944) found an intraerythrocytic parasite in turkey poults. The organism probably belongs in the Babesiidae.

For a more detailed discussion of these diseases, the reader is referred to Chapter 37.

REFERENCES

Banks, W. C.: 1943. *Leucocytozoon smithi* infection and other diseases of turkey poults in central Texas. Jour. Am. Vet. Med. Assn. 102:467.

Campbell, J. G.: 1945. An infectious enteritis of young turkeys associated with *Cochlosoma* sp. Vet. Jour. 101:255.

Herman, C.: 1941. *Plasmodium durae*, a new species of malaria parasite from the common turkey. Am. Jour. Hyg. 34:22.

Kimura, G. G.: 1934. *Cochlosoma rostratum* sp. nov. an intestinal flagellate of domesticated ducks. Trans. Am. Micr. Soc. 53:102.

Kotlán, A.: 1923. Zur Kenntnis der Darmflagellaten aus der Hausente und anderen Wasservögeln Zentralbl. f. Bakt. I. Orig. 90:24.

McNeil, E., and Hinshaw, W. R.: 1942. *Cochlosoma rostratum* from the turkey. Jour. Parasit. (Supplement) 28:349.

——, and Hinshaw, W. R.: 1944. A blood parasite of the turkey. Jour. Parasit. (Supplement) 30:9.

Morehouse, N. F.: 1915. The occurrence of *Haemoproteus* sp. in the domesticated turkey. Trans. Am. Micr. Soc. 64:109.

Rivero, M. D.: 1947. La infección experimental por el *Haemoproteus columbae* Celli y Sanfelice. Rev. Med. Mexicana 26:197.

Travis, B. V.: 1938. A synopsis of the flagellate genus *Cochlosoma* Kotlán, with the description of the two new species. Jour. Parasit. 24:343.

Miscellaneous Diseases

This section includes diseases and conditions which cause considerable financial loss in certain flocks but which are more or less sporadic in nature.

ABSCESS OF THE FOOT PADS

Turkeys sometimes suffer from abscesses of the foot pads (Fig. 41.49). These may resemble corns and are similar to a condition, commonly called bumblefoot, in chickens. The cause is not known, but the abscess probably starts from an infection following an injury to the foot pad. Some of the cases observed have resembled foot rot as seen in other animals. In these instances the affected turkeys had been in

FIG. 41.49 — Abscesses of the foot pads (bumblefoot). (Hinshaw, Univ. of Calif.)

yards that were in constant use and which were covered with several months' collection of feces; cases usually appeared after the fall rains when the yards became very muddy. No doubt many cases of abscesses of the foot pads are also identical with staphylococcal arthritis.

Putting the birds in clean dry quarters and treating the diseased pad will cure many cases. If pus is present, it should be removed, and the area cleaned and treated with an antiseptic healing ointment or tincture of iodine.

Rotating the runs and removing the birds to a clean well-drained yard just before the breeding season are recommended as preventive measures.

ASCITES

Although not a hatchery problem, ascites caused by excess of sodium compounds including common salt (Scrivner, 1916;

Doll et al, 1916) and by exposure to certain disinfectant fumes (Bullis and Van Rockel, 1911) is sometimes confused with omphalitis due to infection described by Brandly (1932). Ascites (dropsy, watery belly, etc.) due to chemical poisoning is seen in young poults or chicks from 2 or 3 days to 2 weeks of age. Excess of salt in the ration and fumes from certain types of disinfectants used for spraying brooder floors are common causes. Excessive salt is usually accidental and may be due to improper mixes or poor screening which allows lumps to get into the mash. The addition of salt to mashes which already contain it because of salted protein concentrates has also been responsible for a few outbreaks. Hatcherymen should warn customers regarding excessive salt in mashes and of the dangers of putting chicks into brooders too soon after spraying the floors with volatile disinfectants.

REFERENCES

Brandly, C. A. 1932. An acute infectious omphalitis of baby chicks. Poultry Sci. 11:279.

Bullis, K. L., and Van Rockel, H.: 1944. Uncommon pathological conditions in chickens and turkeys. Cornell Vet. 34:312.

Doll, E. R., Hull, F. E., and Insko, W. M., Jr.: 1946. Toxicity of sodium chloride for baby chicks. Vet. Med. 41:361.

Scrivner, L. H. 1946. Experimental edema and ascites in poults. Jour. Am. Vet. Med. Assn. 108:27.

BLUEBACK AND CANNIBALISM

Blueback, as the name indicates, is a condition in which the backs of the affected turkeys are discolored blue or black. According to Billings (1940), it is caused

by an injury to the quills of the feathers at the point of entrance into the skin which allows the pigment to escape and tattoo the surrounding skin (Fig. 41.50). Feather picking is the immediate cause, according to him. Exposure to sunlight
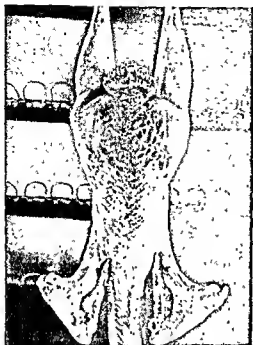
FIG. 41.50 — "Blueback" of turkeys. (Ralston Purina Co.)

after picking is necessary to produce the pigmented condition. Some of the other causes are overcrowding in the brooder, keeping the poults too long on the sun porch, and lack of sufficient fiber in the ration. After picking becomes a habit, the vice is difficult to control, and the financial loss due to lowering of the market grade of the carcass may be considerable.

Another form of cannibalism which often results in evisceration may also be started by feather picking.

Prevention and control consist in correcting the vice. Overcrowding in the brooder should be avoided. Moving poults to the range as soon as picking starts or reducing the numbers in a house are suggested means of control. The feeding of whole oats is recommended by some as another means of prevention.

Mechanical devices are commonly used for the prevention of these vices. Two types are used. The first, inserted in the beak, is patterned after the hog nose ring, in use by swine raisers for prevention of "rooting" These are inserted in one side of the lower mandible (lower beak). A similar type sold by one manufacturer is pinned in the upper beak. The promoters of these nose-guard types claim that turkeys so fitted cannot pick feathers.

Trimming the edges of the beaks will temporarily prevent picking. An electrically heated cauterizing knife has been developed for removing a portion of the upper beak of birds to prevent feather picking and cannibalism. This instrument can be recommended for the prevention of these vices in turkeys. Figure 41.51 from Payne (1956) shows an electrical cauterizing knife used for debeaking and a turkey that has been properly debeaked.

Several ointments are on the market for
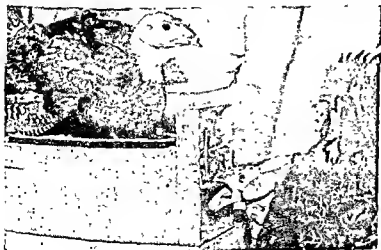
FIG. 41.51 — An electric debeaker in use. The upper beak is removed half way between tip and nostril. (Payne, 1956.)

use on injured birds, principally for prevention of feather picking. These usually consist of a vaseline base, some bitter drug such as aloes, and a red coloring like

carmine. Ewing (1940) suggests an ointment made by mixing 4 ounces of vaseline, ¼ ounce of carmine, and ½ ounce of aloes. Roofing tar is also used.

REFERENCES

Billings, W. A.: 1940. Common diseases of turkeys. Minn. Ext. Bul. 214.
Ewing, W. R.: 1940. Handbook of Poultry Nutrition. W. R. Ewing, Upper Montclair, N.J. P. 205.
Payne, L. F.: 1956. Growing turkeys in Kansas. Kans. Agr. Exper. Sta., Bul. 376 58.

ENTERITIS (NONSPECIFIC) (Inflammation of the Intestines)

Every year numerous immature turkeys die of enteritis from unknown causes. Further research may prove some of these outbreaks to be infectious in nature. At present, however, they must be handled as nonspecific and can probably be attributed to a number of causes.

Stampeding, failure of brooder heaters, sudden changes in the weather, piling in the brooder houses, heat prostrations, sudden changes of feeding methods, and probably, in many cases, faulty feeding methods over a period of several weeks are examples of obscure causes of mortality, with enteritis as the principal pathological manifestation. These various factors may also pave the way for secondary invasion by microorganisms normally of low virulence, which, under such conditions, may cause heavy mortality.

An example of losses starting from an obscure cause that may easily be overlooked follows: Three lots of turkeys about 12 weeks of age were in similar yards where it had been necessary to use an undesirable watering system until a modern drip system was installed. This new system was installed in all three yards at the same time, and the old system removed. Within 48 hours two of the three lots of turkeys became ill, while the third remained normal. On the third day it was discovered that the two groups of sick birds were not drinking the water because of an apparent fear of the new equipment. When the old equipment was replaced in these pens, the birds fought to get at the water and drank three or four times as

much as normal for the day. Only 1 per cent of the birds died before and within 24 hours after the discovery of the cause, but there was a distinct difference between the two affected lots and the third lot for nearly a month. A difficulty usually experienced in making a diagnosis for such outbreaks is the lack of sufficient history.

Losses are commonly experienced by turkey growers following the transfer of poults from the starting brooders to the "cooling" brooders. A common practice is to rear poults in small units in battery types for 3 to 4 weeks, and transfer from these to regular brooder houses with yards or wire porches. Losses following such transfers are usually due to failure to use the same type of feeders and waterers as in the starting brooders. Use of similar equipment and careful watching of the poults to see that they start eating and drinking properly after such moves will avoid losses.

Signs. The principal signs are loss of appetite, a tendency to separate from the well birds, diarrhea, and a general haggard appearance. Temperatures are usually normal or subnormal. The birds may sit in a listless manner with their heads hung or turned up over their backs. On open ranges, where the majority of the flock is affected, difficulty is often experienced in keeping the birds under control; the turkeys appear nervous and may wander for hours, often straying ½ mile or more from the main camp. During the course of the disease, often a period of several weeks, a marked loss of flesh may occur. The mortality is not, as a rule, high for a single

day; but over a period of 3 or 4 weeks, 25 per cent or more of the birds may die.

Necropsy findings. Emaciation and enteritis, varying from a catarrhal to a more advanced inflammatory type, are the principal necropsy findings. The heart is usually flabby. The blood, in many instances, fails to clot for several hours after death; it is usually very dark in appearance. The liver often appears congested, and dark venous blood oozes from cuts made on its surface. In many respects the symptoms and necropsy findings resemble those of acute poisoning.

Prevention, control, and treatment. It is extremely difficult to give methods of prevention, control, and treatment for enteritis of an unknown cause. Every effort should be made to determine the cause before treatment with drugs is planned. Exploratory trials with the new drugs, rather than total flock treatment, is recommended until the efficacy of a particular drug is determined.

Sound, rational turkey husbandry is probably the best preventive. An adequate diet and an ample supply of pure, fresh water are important. Avoiding the possible causes of enteritis is essential. A few have already been mentioned, and others will suggest themselves. Any abnormality that will cause the bird to lose its appetite or develop an intestinal disturbance, even for a few days, may cause heavy losses for several weeks.

Sudden changes of feed should be avoided, but if the flock is definitely not doing well on a particular diet, the reason should be sought. If the feed is responsible, a gradual change to another method should be made. If the original method is resumed after the birds have recovered, the shift should also be gradual.

Turkeys that are to be taken off a full-feed ration and transferred to a grain field should be fed some of the same type of grain as that grown in the field for a week or two before being moved to the range. This procedure accustoms them to the new grain and will prevent a setback and

possible heavy losses In addition, for a few days after they are moved to the range, the birds should have some of the mash previously used.

ENTERITIS (HEMORRHAGIC)

Pomeroy and Fenstermacher (1937) describe a hemorrhagic enteritis in turkeys ranging from 7 to 12 weeks of age. The disease appeared in Minnesota during the summer months and caused a mortality of about 10 per cent. The flocks involved in this outbreak had been reared on wire porches for 6 to 8 weeks and were then transferred to field ranges. The losses occurred from 10 to 14 days after the poults were put on range. The ranges were very poor that year, greens were not available in sufficient quantities, and there was a distinct lack of sunshade and shelter. *Escherichia coli* and an unidentified Gram-positive rod were the only bacteriological findings. Neither of these was proved to be the causal factor.

A condition similar to that reported by Pomeroy and Fenstermacher has been observed in a few instances by Hinshaw. In these outbreaks the losses have always occurred a few days after transferring the poults to ranges or to yards adjoining the range brooder. In two such outbreaks the range contained a young succulent growth of alfalfa, and in a third the yard was overgrown with weeds and grasses including some sweet clover. Losses stopped in all three instances when the poults were returned to the brooders and were put back on a dry mash ration. Later they were put back on the range for short intervals at first and finally for the full period and without further losses. The causal factors were not determined. These findings constitute additional reasons for use of great precaution when moving poults from one environment to another.

Gale and Wyne (1957) describe two outbreaks of hemorrhagic enteritis in 7. to 11-week-old turkeys. Both occurred in turkeys being reared in confinement. Bacteriological examination revealed *Escher-*

ichia coli infection in the livers of about 20 per cent of the poults. No evidence of a viral infection could be established. No mention is made by them of attempts to isolate toxic fungi such as described by Forgacs and Carll (1955) and Forgacs *et al.* (1958) as a cause of a similar condition in chickens. Toxic fungi should not be overlooked as a possible cause of hemorrhagic enteritis in turkeys. Poisoning due to overdose of drugs (Sanger *et al.,* 1956) should not be overlooked. Toxic substances such as beat-aminopropionitrile

(BAPN) (Barnett *et al.,* 1957; Pritchard *et al.,* 1958) and vitamin K deficiency should also be considered in making a diagnosis.

The condition described as aortic rupture (dissecting aneurism) in turkeys by McSherry *et al.* (1954), Carnaghan (1955), Gibson and DeGruchy (1955), and Pritchard *et al.* (1958) should not be confused with hemorrhagic enteritis.

See prevention, control, and treatment under enteritis (nonspecific), above.

REFERENCES

Barnett, B. D., Richey, D. J., and Morgan, C. L.: 1957. Hemorrhage in chicks induced by beta-aminopropionitrile and sulfaquinoxaline. Poultry Sci 36:1104.

Carnaghan, R. B. A.: 1955. Atheroma of the aorta associated with dissecting aneurysm in turkeys. Vet. Record 67 568.

Forgacs, J., and Carll, W. T.: 1955. Preliminary mycotoxic studies on hemorrhagic disease in poultry. Vet. Med. 50:172.

——, Koch, H., Carll, W. T., and White-Stevens, R. H.: 1958. Additional studies on the relationship of mycotoxicoses to the poultry hemorrhagic syndrome. Am. Jour. Vet. Res. 19.744.

Gale, C., and Wyne, J. W.: 1957. Preliminary observations on hemorrhagic enteritis of turkeys. Poultry Sci. 36:1267.

Gibson, E. A., and DeGruchy, P. H.: 1955. Aortic rupture in turkeys subsequent to dissecting aneurysm. Vet. Record 67.658.

McSherry, B. J., Ferguson, A. E., and Ballantyne, J.: 1954. A dissecting aneurism in internal hemorrhage in turkeys. Jour. Am. Vet. Med. Assn. 124:279.

Pomeroy, B. S., and Fenstermacher, R.: 1937. Hemorrhagic enteritis in turkeys. Poultry Sci. 16 378.

Pritchard, W. R., Henderson, W., and Beall, C. W.: 1958. Experimental production of dissecting aneurysm in turkeys. Am. Jour. Vet. Res. 19.696.

Sanger, V. L., Yacowitz, H., and Moore, E. N.: 1956. Micropathological changes in an experimental hemorrhagic syndrome in chickens fed sulfaquinoxaline and suggested cause of the disease. Am. Jour. Vet. Res. 17.766.

AORTIC RUPTURE (Dissecting Aneurism)

Internal hemorrhage caused by aortic rupture or dissecting aneurism has become an important cause of mortality in turkeys. The first report of the disease was made by Dorrell *et al.* (1952). They reported its presence in many of the United States. McSherry (1954) described losses in Canada that occurred also in 1952. Further evidence of the widespread occurrence are the reports from Great Britain by Carnaghan (1955) and Gibson and DeGruchy (1955). McSherry *et al.* (1954) and Pritchard *et al.* (1958) describe the disease in detail.

The etiology has not been clearly established. A number of investigators including Barnett *et al.* (1957), Pritchard *et al.*

(1958), and Waibel and Pomeroy (1958) have been able to artificially produce aortic rupture by feeding small quantities of beta-aminopropionitrile (BAPN) a toxic factor found in sweet-pea seeds. Since sweet-pea seeds are not used in turkey rations this source is unlikely, and no other source has yet been reported.

High blood pressure, induced by any one of a number of stress factors is considered as a contributing cause (Ringer, 1961). Ringer (1962) also considers genetic inheritance of a defective blood vessel as a possible factor. Atherosclerosis, predisposed by inheritance, has also been proposed by Ringer.

Aortic ruptures occur under field condi-

tions in rapidly growing birds and mortality is highest in males. Losses occur most often in birds from 10 to 16 weeks of age.

Clinical signs are seldom seen, and the owner first realizes his problem when he discovers a dead bird showing a typical blanched head. Occasionally death is preceded by gasping and by blood running from the mouth.

On necropsy the abdominal cavity is seen to be filled with blood due to the massive hemorrhage. No evidence of trauma is seen. Rupture of the posterior aorta due to a typical dissecting aneurism is most often

seen at a location in the region between the kidneys. Blood may be found in the air sacs, lungs, and even in the mouth and crop.

A number of remedies have been suggested, but the tranquilizer, reserpine, is most commonly used. Its real value remains to be determined. It is given in the feed and recommendation of the manufacturer should be observed.

Reviews on the use of reserpine in turkeys will be found in papers by Craig et al. (1962), Speckman and Ringer (1962), and Krista et al. (1963).

REFERENCES

Barnett, B. D., Bird, H. R., Lalich, J. J., and Strong, F. M.: 1957. Toxicity of beta-aminopropionitrile for turkey poults. Proc. Soc. Exptl. Biol. Med. 94:67

Carnaghan, R. B. A.: 1955. Atheroma of the aorta associated with dissecting aneurysm in turkeys. Vet. Record 67:568.

Craig, F. R., Blow, W. L., Monroe, R. J., and Barber, C. W.: 1962. The influence of reserpine on late growth of turkeys. Poultry Sci. 41:711.

Dorrell, W. B., Pomeroy, B. S., Carr, W. S., and Jerstad, A. C.: 1952. Discussion Proceedings Book, Am. Vet. Med. Assn. 1952:280.

Gibson, E. A., and DeGruchy, P. H.: 1955. Aortic rupture in turkeys subsequent to dissecting aneurism. Vet. Record 67:650.

Krista, L. M., Burger, R. E., and Waibel, P. E.: 1963. The influence of various drugs on the growth and beta-aminopropionitrile-induced dissecting aneurysm of turkeys. Poultry Sci. 42:522.

McSherry, B. J., Ferguson, A. E., and Ballantyne, J.: 1954. A dissecting aneurism in internal hemorrhage in turkeys. Jour. Am. Vet. Med. Assn. 124:279.

Pritchard, W. R., Henderson, W., and Beall, C. W.: 1958. Experimental production of dissecting aneurysms in turkeys. Am. Jour. Vet. Res. 19:696.

Ringer, R. K.: 1961. The effect of beta-aminopropionitrile on the blood pressure of turkeys. Poultry Sci. 40:1001.

———: 1962. What we know about internal bleeding. Turkey World 37 (May):32.

Speckman, E. W., and Ringer, R. K.: 1962. The influence of reserpine on plasma cholesterol, hemodynamics and arteriosclerotic lesions in the broad breasted bronze turkey. Poultry Sci. 41:40.

Waibel, P. E., and Pomeroy, B. S.: 1958. Studies on the production of aortic hemorrhage in growing turkeys with beta-aminopropionitrile. Poultry Sci. 37:934.

HEAT PROSTRATION (Heat Stroke)

Heat prostration is usually associated with high humidity accompanying high temperatures or with very low humidity on excessively hot days. Stiles (1943) reported a case of heat exhaustion in a flock of 3 week-old turkey poults which were abruptly transferred from cool turkey brooders to quarters where the heat inside the building and on the sun porches was extremely hot. Wilson and Woodard (1955) found that air temperatures above 90° F. cause hyperthermy in turkeys and that the body temperature is definitely

influenced by the amount of shade at such temperatures.

The symptoms are labored breathing, weakness, excessive thirst, and high temperature, followed by complete prostration. Losses can be prevented by furnishing ample shade facilities, especially for the poults just transferred from the brooder house to an open yard or range. If a house is available on the range, the young poults may well be sheltered in it during the hottest part of the day, but with all the wintest part of the day, but with all the windows open for ample circulation of air. As soon as the poults become accustomed

to the new quarters, they will stay inside during the excessive heat; water and feed should be left both inside and outside the house for the first few weeks. Out of doors, trees make the best shade; but an abundance of cheap artificial shade can be made from old lumber and posts. Thatched roofs may be used advantageously if material for covering the shelter can be secured. Pure, fresh water must be available at all times. It should be kept in a shady place and in enough containers so that the birds will have no difficulty

in getting to it. If, in spite of all precautions, turkeys are overcome by the heat, they should be put in a shady, well-protected place and sprayed with cold water. Used in time, this procedure will save a large number. Filling the crop with cold water by means of a rubber tubing and a funnel is also advisable. Dipping the birds in cold water may be effective, but care must be taken to prevent drowning. As they may remain weak for several days, they should be kept in the shade with food and water easily accessible.

REFERENCES

Stiles, G. W.: 1943. Heat exhaustion in young turkeys. Poultry Sci. 22:242.
Wilson, W. O., and Woodard, A.: 1955. Some factors affecting body temperatures of turkeys. Poultry Sci. 34:369.

INJURIES

Injury to the female by the male. Severe losses occur in many breeding flocks because of the females' backs being badly torn by the male during the mating process (Fig. 41.52). Badly torn females seldom recover sufficiently to produce fertile eggs during the remainder of the season; and if the wound does heal, the area is tender and easily torn when the bird is trodden again.

Some males are much more vigorous and rough in the mating than others, and many of the losses can be traced to one or two individuals in a flock. These males should immediately be *replaced* by reserves. One method of prevention is the removal of the toenails from the males (Fig. 41.53A and B) This should be

done about a week before the males are put into the breeding pens. A convenient instrument for removing the toenails is a pair of pruning shears of the roll-cut type shown in Figure 41.53A. An electric soldering iron or some other form of a searing iron can be used for searing the cut surface to stop hemorrhage.

Another method of preventing breeding females from being torn is to fit a canvass jacket over the back (Figs. 41.54 and 41.55). These jackets, which can be purchased at a reasonable price, are recommended for general use. Care should be taken to purchase saddles which fit correctly in order to prevent strangulation or injury to the body or wings.

If an injured hen is discovered im-

FIG. 41.52 — Turkey hen with a severe laceration caused by a male during the mating process. (Hinshaw, Univ. of Calif.)
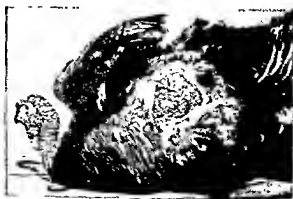
FIG. 41.53 — (A) A method of trimming the toenails of a male turkey to prevent injuries during the mating season. (B) Feet of a male turkey after trimming the toenails as illustrated in A. (Hinshaw, Univ. of Calif.)

mediately, the torn edges of skin should be sutured. The bird should then be placed in a pen where there are no males and left for about 2 weeks. An antiseptic dusting powder will induce healing and prevent attacks by flies. As soon as the wound begins to heal normally, the hen can be fitted with the canvas jacket described above and can be returned to the breeding pen. She should be carefully watched, however, and if again injured

FIG. 41.54 — A type of "apron" or "saddle" in common use for prevention of injuries to females during the mating season. See Figure 41.55. (Hinshaw, Univ. of Calif.)

by the males, should be returned to the isolation pen.

Where several males are in one pen, the transfer of a male to a pen of injured females may be a better procedure than putting the injured hens back in the regular pen. The time required for complete recovery depends on the extent of the injury and the efficiency of the treatment. Whether or not treatment is worth-while depends on the value of the individual and the time available.

Injuries from fighting. As males are more likely to be injured from fighting than are females, often a valuable male should be separated from its penmates if it is not able to defend itself successfully. A male which has been away from the flock for any length of time or which has just been purchased must be protected when placed with other males, because they will invariably fight it.

Minor injuries seldom require treatment and will heal readily if the bird is unmolested. Severe lacerations about the head usually respond to an antiseptic dusting powder.

Miscellaneous injuries. Injuries from being caught in fences, from flying into objects during stampedes, from rough handling, and from many other causes are cared for in much the same manner as injury by a male.

Wickware (1945) reported that grasshoppers may caused death of turkeys by mechanically injuring the walls of the

FIG. 41.55 — Turkey hen with the "saddle" shown in Figure 41.54 in place. (Hinshaw, Univ. of Calif.)

crop and intestine In some of these cases the walls of these organs were punctured by grasshopper legs He suggests that such losses can be prevented by feeding plenty of mash to turkeys that have access to ranges where grasshoppers are abundant.

Dickinson and Clark (1946) and Bullis and Van Roekel (1944) have reported brooder stove residue burns on the heads of turkey poults that were brooded under stoves heated with gas briquets or kerosene. The injuries in these cases are similar and range from mild burns to dry gangrene Dickinson and Clark state that the use of tight-fitting stove pipe joints will prevent seepage of the oily residue

responsible for gas briquet type burns. Attention paid to kerosene or similar burners to prevent leakage of oil will stop losses from such causes.

A type of injury seen now and then is shown in Figure 41.56. The bird is usually found with its head hanging downward and forward and is unable to change this position. The neck muscles are much swollen and are hot to the touch. Often one will find a tuft of feathers pulled from the side of the neck and evidence of a bruise. Dislocation of a vertebra or fracture of a vertebral process has been found to be the cause in most cases. In at least two, the injury resulted from entanglement in a wire fence during attempts to reach feed. Correction of the dislocation by massage and tension gave relief and complete recovery in about 2 weeks. Other cases have taken from 3 to 6 weeks to recover but have shown no detrimental aftereffects. If such an injury is found in a flock, the cause should be determined. Any dislocation found should be corrected. Until recovered the bird should be isolated but placed near water and feed containers.

FIG. 41.56 — Posture of turkey suffering from a slight dislocation of one vertebra of the neck. The bird could not raise its head, and the muscles of the area were severely swollen. (Hinshaw, Univ. of Calif.)

REFERENCES

Bullis, K. L., and Van Roekel, H.: 1944 Uncommon pathological conditions in chickens and turkeys Cornell Vet 34.312

Dickinson, E. M., and Clark, W G.: 1946. Brooder stove-residue burns on turkey poults. Cornell Vet. 36:314.

Wickware, A. B. 1945 Grasshoppers, a potential danger to turkeys. Canad. Jour. Comp. Med. 9 80

KERATOCONJUNCTIVITIS
(Blepharoconjunctivitis)

Bierer (1956, 1958) describes a disease characterized by inflammation of the eyelids and, in more advanced stages, an ulceration of the anterior portion of the eyeball. According to him, the disease generally is found in adult turkeys in the early breeder flocks — November to January. Hens appear to be more often affected than toms. Economic losses may be severe from high mortality, loss in egg production, and reduced fertility. The market value of the affected birds is lowered.

Signs. Bierer describes the following signs of disease. The first evidence is a whitish foam at the inner corner of the eye near the nictitating membrane. One or both eyes may be affected. A whitish crust may be seen on the outer surface of the eyelid. Excessive lacrimation occurs when the bird begins to rub the affected eye on its feathers. The conjunctiva becomes progressively more inflamed, caseous exudate appears, and finally the lids become encrusted, preventing vision. Ulceration of the cornea, destruction of it, loss of aqueous humor from the anterior chamber, and
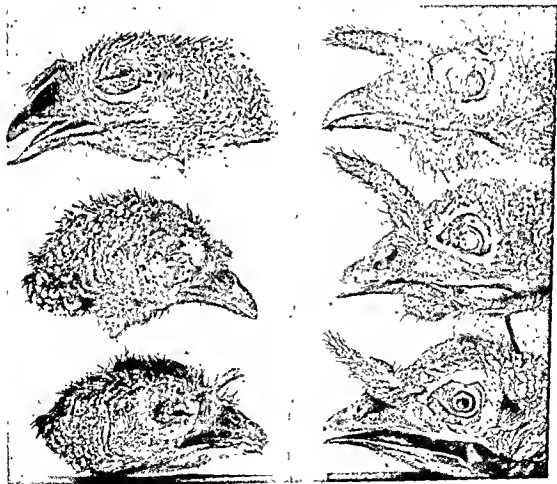
FIG. 41.57 — Keratoconjunctivitis. Left: (upper) severe conjunctivitis; (middle) thickened eyelids and conjunctival sac filled with cheesy matter; (lower) eyelids removed to show the presence of the cheesy matter. Right: (upper) ulceration of the cornea, eyelids removed; (middle and lower) severe keratitis with extensive destruction of corneal tissue, eyelids removed. (Bierer, 1956.)

permanent blindness are the terminal phases of the disease. Death may result from inability to obtain adequate feed and water. Actual mortality in the flock usually is low, but the morbidity may reach 35 to 40 per cent of the flock. The greatest economic loss is from loss in egg production, poor fertility, and lowered market value of the affected birds. Figure 41.57 shows various stages in the development of the lesions.

Etiology. The etiology is obscure. Bierer (1958) presents convincing evidence that deficiency of vitamin A plays an important role probably as a stress factor. Secondary infections probably account for many of the pathological changes although Bierer was unable to incriminate any single microorganism.

Differential diagnosis. There are a number of reports on eye involvements in turkeys that must be differentiated from this disease. Included are Manson's eyeworm disease (Schwabe, 1950); Plasmo-

dium lophurae infection (Becker et al., 1949); aspergillosis (Moore, 1953); salmonellosis (Evans et al., 1955); paracolon infections (Hinshaw and McNeil, 1946); and granulomatous chorioretinitis (Saunders and Moore, 1957). Similar signs and lesions are sometimes seen in turkeys that have been vaccinated against fowl pox at an early age and exposed several months later after the initial immunity has been reduced or lost. These cases usually develop in old breeders following fighting. In such cases the causative virus can be easily isolated and demonstrated as the cause.

Prevention and control. Bierer suggests prompt isolation of the affected birds to avoid cannibalism by mates. Individual treatment following surgical cleaning of the area with antibiotic ointment has given good results. Administration of vitamin A to each bird is indicated. Supplementing the ration with feed containing a high vitamin A level is suggested by Bierer as a preventive measure.

REFERENCES

Becker, E. R., Brodine, C. E., and Marousek, A. A.: 1949. Eyelid lesion of chicks in acute dietary deficiency resulting from blood induced *Plasmodium lophurae* infection. Jour. Infect. Dis 85:230.

Bierer, B W: 1956 Keratoconjunctivitis in turkeys: A preliminary report. Vet. Med. 51:363.

——: 1958. Keratoconjunctivitis in turkeys: II. The relationship of vitamin A, infectious agents and environmental factors to the disease. Vet. Med. 53:477.

Evans, W. M., Bruner, D. W., and Peckham, M. C.: 1955. Blindness in chicks associated with salmonellosis Cornell Vet. 45,259.

Hinshaw, W. R., and McNeil, E.: 1946 The occurrence of type 10 paracolon in turkeys, Jour. Bact. 51:281.

Moore, E. N.: 1953. *Aspergillus fumigatus* as a cause of ophthalmitis in turkeys. Poultry Sci. 32,796.

Saunders, L. Z., and Moore, E. N: 1957 Blindness in turkeys due to granulomatous chorioretinitis Avian Dis. 1.27.

Schwabe, C. W. 1950 The tropical eyeworm of poultry Am. Jour. Vet. Res. 11:286

OMPHALITIS

Omphalitis, or navel infection, is characterized by failure of the navel opening (umbilicus) to close properly, with resultant infection of the internal organs. The disease can often be traced to faulty incubation or to hatchery insanitation. In most instances the poults are weak when removed from the incubator, and losses may start before time for shipment from the hatchery.

Published reports of the disease include those of Volkmar (1929), Brandly (1932). and Williams and Daines (1942). Williams and Daines associated a severe outbreak of impetigo staphylogenes among hatchery workers with a concurrent outbreak of omphalitis in poults in the same hatchery.

The signs are general weakness, lack of body tone, and a tendency to huddle. In the brooder the poults appear cold and stay under the hover. When handled they

feel flabby, the abdomen is enlarged, and they do not have the firmness of a normal poult. The navel opening, which usually is completely healed within 72 hours, is inflamed, moist, and fails to close for several days. Often a definite scab forms over the opening. The course is rapid, death often occurring within a day after signs are noted; the mortality is high, often reaching 50 per cent of the brood. The course of the disease and the mortality depend on the type of microbial flora that exists in the hatchery and on the promptness of initiating preventive measures.

On necropsy, edema of the muscles of the abdomen and breast, an unabsorbed yolk, and peritonitis are the principal observations. The contents of the retained yolk are usually more liquid than normal, and rupture of the yolk sac is common.

The disease is probably a result of a

mixed infection of hatchery origin. In the outbreaks reported to the writer, thorough cleaning and disinfection of the hatchery rooms and incubators have prevented further losses. The formaldehyde fumigation method, outlined under the section on disinfectants (page 131) will eliminate the disease from the hatchery. Insko et al. (1941) and Insko (1949) suggest that two to three times the amounts of formalin and potassium permanganate be used to fumigate incubators known to be spreading omphalitis. This strength should be used between hatches. Incubator rooms and all hatchery equipment as well as the incubators should be fumigated. (See section on formaldehyde fumigation, page 132.)

No remedy or adequate method of controlling the disease in the brooder has been found. Keeping the poults comfortable and applying hygienic measures will help reduce the mortality to a minimum.

REFERENCES

Brandly, C. A.: 1932. An acute infectious omphalitis (inflammation of the navel) in baby chicks. Poultry Sci. 11:279.
Insko, W. M.: 1949. Physical factors in incubation. In Taylor, L. W., Fertility and Hatchability of Chicken and Turkey Eggs. J. Wiley and Sons, New York, p. 209.
———, Steele, D. G., and Hinton, C. M.: 1941. Effect of formaldehyde fumigation on mortality of chick embryos. Ky. Agr. Exper. Sta. Bul. 416.
Volkman, F.: 1929. Omphalitis in baby chicks and poults. Jour. Am. Vet. Med. Assn. 75:647.
Williams, R. B., and Daines, L. L.: 1942. The relationship of infectious omphalitis and impetigo staphylogenes in man. Jour. Am. Vet. Med. Assn. 101:26.

PENDULOUS CROP

Serious losses from pendulous crop (Fig. 41.58A) in some flocks are, according to Hinshaw and Asmundson (1936) and Asmundson and Hinshaw (1938), the result of a hereditary predisposition toward the condition. Turkeys with the inherent weakness develop pendulous crops after the increased liquid intake that follows the first wave of excessively hot weather. The crop, once expanded, seldom returns to normal size, especially if the hot, dry weather continues. It may contract for a few days, if the weather becomes cool, and then expand again during the next hot spell. Although a few birds recover, the majority continue to have pendulous crops. In this condition the crop does not empty normally; stagnant, sour liquid contents are

retained in the bulbous portion. As time goes on, the mucous membrane thickens and may become ulcerated (Fig. 41.58B) due to secondary microbial infections.

The appetite is not greatly affected, but digestion is hindered. The feed and water remaining in the crop may increase until the crop and its contents equal one-fourth of the total live weight of the bird. The bird may continue to grow, but will remain unthrifty and may become emaciated.

Pendulous crops caused by an inherent weakness must be distinguished from similar conditions that sporadically result from impactions, mycosis, trichomoniasis, and other crop infections. These conditions, however, may be exaggerated in flocks having a hereditary tendency towards pendulous crops.

Studies by Rigdon et al. 1958) and Man-
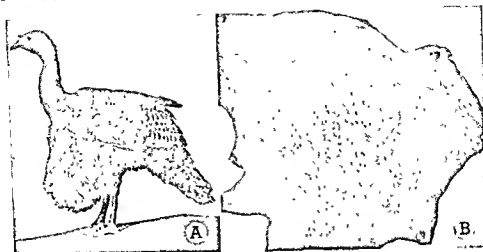
FIG. 41.58.—(A) An 8-month-old female turkey with a pendulous crop of about 5 months' duration. (B) Section of a pendulous crop showing thickening and ulceration of the mucous membrane. (Hinshaw, Univ. of Calif.)

ire et al. (1958) indicate that the normal diets fed turkeys do not greatly influence the incidence of pendulous crops in a flock. They, however, experimentally produced pendulous crops in turkeys by feeding a ration containing cerelose as a substitute for starch. When such birds were returned to a starch ration, the pendulous crops regressed. No mention is made as to whether the turkeys used were from parent stock having a hereditary tendency to this condition. They also found that *Saccharomyces tellustris* grows and multiplies rapidly in crops of turkeys fed cerelose. This yeast produces large amounts of gas which is thought to be the cause of the expansion of the crops of cerelose-fed birds. *Candida albicans* was also found to be a common inhabitant of the crops of the turkeys used in the cerelose experiments, but it did not multiply when cerelose was fed, and no frank cases of candidiasis developed.

Course, mortality, and causes of death. The course of the disease is chronic; as mentioned above, very few birds recover even with treatment. Some live for as long as 2 years, but the mortality of the affected birds in a flock may exceed 50 per cent.

The causes of death are (1) rupture of the crop by the bird's toes in its attempt to walk or run, (2) mechanical pneumonia from the seepage of crop contents into the bronchi during mechanical efforts to drain the crop or as a result of a backflow when the bird lowers its head, and (3) starvation due to insufficient intake of food or to improper digestion.

Necropsy findings. Necrotic ulcers, varying in nature according to the type of the contents and severity of the case, frequently occur. Scraping the necrotic membrane from the surface leaves a denuded, bleeding area. This type of necrosis is distinguished from that seen in trichomoniasis by the tendency of the latter to form individual pyramidal ulcers (Fig. 41.47) as compared with the diffuse, spreading nature of the former. Demonstration of trichomonads furnishes a further means of differentiation. In a few cases, lesions typical of moniliasis (thrush) (Fig. 41.12) have also been observed. In these cases fungi are readily demonstrated. The contents of the crops have varied from a watery, sour-smelling mass to a solid bolus of mud, feces, and grain. Semiliquid contents have been most common. The contents usually suggest a depraved appetite.

Few or no changes in any organ except the crop and possibly the lower esophagus are seen on necropsy. The mucous mem-

brane of the bulbous portion of the crop is thickened and in folds. Areas of diseased lung tissue varying in size are easily seen in those cases where the cause of death has been mechanical pneumonia caused by the entrance of crop contents into the lung. In such cases, food particles are found in the bronchi when the latter are carefully dissected. The air sacs are sometimes involved, and foreign matter can be seen when scrapings from them are examined microscopically.

Prevention, control, and treatment. Since pendulous crops are usually associated with a hereditary weakness, the best preventive measure is to avoid mating any birds that have a family history of this weakness. Although this is a difficult procedure in the flock that is not trapnested, much can be done to prevent the condition from becoming established. Poults with affected crops should be caught and toe-marked or banded so that they can be eliminated at the time turkeys are selected for breeding.

Sufficient shade during the hot months will reduce the numbers of pendulous crops in a flock. It is doubtful, however, whether any procedure other than eliminating the inherent tendency will remove the possibility of having a few cases.

Many methods for "curing" pendulous crops have been described by turkey growers. These have included various operations, the use of cloth vests or supporters, and methods of portioning out the water supply to the affected birds. Most of the methods have produced few actual recoveries.

Removing a portion of the crop surgically results in a high percentage of recoveries, but the time consumed probably does not warrant the procedure as a routine practice. Washing out the crop with warm water containing a weak antiseptic and then tying off a portion of the skin over the enlarged crop will yield temporary relief until market time. If only a few cases appear, it is more economical to kill the affected birds

REFERENCES

Asmundson, V. S., and Hinshaw, W. R.: 1938. On the inheritance of pendulous crop in turkeys (*Meleagris gallopavo*). Poultry Sci. 17:276.

Hinshaw, W. R., and Asmundson, V. S.: 1936. Observations on pendulous crop in turkeys. Jour. Am. Vet. Med. Assn. 88:154.

Manfre, A. S., Wheeler, H. O., Feldman, G. L., Rigdon, R. H., Ferguson, T. M., and Couch, J. R.: 1958. Fungi in the crop of the turkey. Am. Jour. Vet. Res. 19.688.

Rigdon, R. H., Ferguson, T. M., Feldman, G. L., Wheeler, H. O., and Couch, J. R.: 1958. A study of the mechanism of the experimentally induced pendulous crop in the turkey. Am. Jour. Vet. Res. 19.681.

POISONING*

Although losses from poisoning in turkey flocks are not great, a few cases are briefly described below. The tolerance of turkeys to rodent poisons is also discussed in answer to inquiries on this subject. In most outbreaks traced to poisoning, the signs and necropsy findings resemble those already described under the heading of enteritis. The diagnosis depends on discovering poison by chemical analysis of the crop or gizzard contents or on finding poison in the food supply.

Arsenic. DeLay (1910) reported losses in

10-week-old poults from eating grasshopper bait containing sodium arsenite and bran moistened with water. The bait was spread unevenly on a turkey range so that the birds had access to clumps of the mixture as well as to the grasshoppers. The owner reported a mortality of 5 per cent from the poisoning. DeLay fed some of the same mixture to 8-week-old poults in such a manner that the poults consumed from 0.25 to 0.5 gram of arsenic trioxide. Both dosages killed the experimental poults; the larger dose in 2 to 12 hours, and the smaller dose in 20 to 72 hours after being fed. The smaller dosage approximated that consumed on the ranch. The postmortem findings in the field cases

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* See also Chapter 40.

described by him were grasshoppers in the crop, hemorrhagic inflammation of the duodenum and jejunum, and a "sweetish" odor of the gizzard and intestinal contents Arsenic was detected in the intestinal contents and in the grasshoppers found in the crop by the Gutzeit method.

According to Whitehead (1931) arsenic in bran used for grasshopper poisoning is not present in sufficient amounts to produce mortality in birds if the mixture is spread evenly and thinly over the ground Growers are cautioned, however, to be sure that these recommendations are followed if such a procedure is necessary on a turkey range

Copper sulfate (bluestone). According to experimental work by Hinshaw and Lloyd (1931), turkeys may be poisoned by copper sulfate added to the drinking water in concentrations greater than 0.2 per cent (1 500 dilution). As turkeys do not like copper sulfate solutions in any dilution and will avoid them if untreated water is present, poisoning is unlikely unless no other source of drinking water is available. In cool weather turkeys may go without drinking for several days rather than drink water containing even nontoxic doses of this chemical. For these reasons, copper sulfate is not recommended except for specific uses and in concentrations not exceeding 0.05 per cent (1:2,000 dilution). The poisoning is usually evidenced by a greenish-blue stain on the crop. Marked erosion of the mucous membranes follows excessive doses

Mercuric chloride (corrosive sublimate). Mercuric chloride is well known for its toxic nature, but in spite of this is often carelessly used on turkey ranches as a disinfectant and remedy. It is too commonly recommended for treatment of drinking water without experimental basis for the recommendation.

A series of trials made on the toxicity of this chemical for turkeys by McNeil and Hinshaw (1945) showed that a 1:4,000 dilution as a sole source of drinking water was toxic. The principal necropsy finding in mercuric chloride poisoning was a marked thickening and necrosis of the gizzard lining. There was some escharotic thickening in the crop, and the mucous membrane of the proventriculus was often sloughed.

Poisonous weeds. The fact that turkeys are often ranged among poisonous weeds suggests the reason for losses that are sometimes experienced on pasture lands. There are no experimental data available, however, on weed poisoning in turkeys. Where heavy losses occur in young turkeys reared on pasture, poisonous weeds should be sought as a possible cause. Suspected plants should be sent to a diagnostic laboratory together with tissue specimens for diagnosis and identification.

As a rule, animals or birds will not eat poisonous plants, unless other forms are not available. Most cases of poisoning result from the eating of young, growing shoots that come up in the spring before more palatable and nonpoisonous plants appear. Under certain conditions the seeds of poisonous plants may cause losses if accidentally mixed with grains.

The only method of control is to remove the cause. If the birds are ranging in suspected areas, confining them in enclosures for a few days and supplying them with sufficient freshly cut greens is recommended. When they are again turned out on the range, the supply of fresh greens should be continued until the suspected poisonous plants have been replaced by nonpoisonous varieties.

Examples of poisonous weeds which have been known to cause losses in turkeys are the seeds of certain of the lupine, young shoots of oleander, and the second, succulent growth of Sudan.

Oleander (Nerium oleander) poisoning in turkeys is occasionally seen, but under normal feeding conditions poults will not eat even the young succulent shoots. In one experimental trial by McNeil and Denny (1939), five out of six 4-week-old poults were killed by inserting leaves of oleander sprouts into their crops. The same poults had refused to eat the leaves when offered as greens. The poults died

within 24 hours after the forced feeding, and at necropsy showed hemorrhagic enteritis. Three adult turkeys were fed young succulent oleander shoots for 2 weeks in lieu of greens. The birds continually refused them, even when they were cut up and mixed with the grain. The presence of oleander leaves in the crop and gizzard, together with a history of the poults eating the plant, is evidence of poisoning.

Sodium bicarbonate (baking soda). Sodium bicarbonate has been shown by several investigators (Delaplane, 1934; Hoffman, 1942; and Witter, 1936) to cause losses in chickens. These losses are manifested by lesions in the kidneys and other organs similar to those seen in gout. Hoffman found that the continuous use of amounts of sodium bicarbonate in excess of 15 grams per gallon of drinking water is toxic for baby chicks if used as a substitute for all other drinking water.

The toxicity of sodium bicarbonate for turkey poults from 4 to 8 weeks of age has been determined at the California station (unpublished data). The results obtained were similar to those reported by the above investigators. When more than 0.6 per cent of sodium bicarbonate was given in the drinking water to 4- and 6-week-old poults, some mortality resulted, while 8-week-old poults were able to tolerate 1.2 per cent. Marked uremia and arthritis developed in all ages when over 0.6 per cent was given. As noted by Witter, sodium bicarbonate given in subtoxic doses also caused increased water consumption and diarrhea in turkey poults. Therefore, sodium bicarbonate is not a safe drug to use on the turkey ranch.

Sodium chloride (common salt). One outbreak of enteritis in turkeys about two-thirds grown finally proved to be associated with the use of well water containing a high percentage of common salt. This was the only source of water, and the losses probably resulted from heat prostration combined with salt dehydration; the turkeys did not like the water and drank only small quantities. A supply of fresh

water stopped the losses within a few days. Another instance of losses from enteritis probably due to salt consumption was traced to boxes of salt placed on the range for sheep that were being pastured with the turkeys (See material on ascites, page 1344).

Strychnine. Inquiries on possible poisoning by the strychnine coated grain used for rodent control on cut-over grain fields stimulated a series of experiments to determine the tolerance of turkeys for strychnine. Based on the results, turkeys will tolerate the usual amounts of strychnine in poisoned grain. Despite considerable variation in individual tolerance, there is probably little danger, provided other grain is available. Turkeys dislike grain coated with even minute amounts of strychnine and, after the first taste, will usually leave the planted poison bait alone and seek more palatable food.

Miscellaneous. Many other poisons could be mentioned, but they are not common causes of losses, and little is known about the exact tolerance of turkeys to them. Circumstantial evidence often points to poisoning when it is difficult to prove that a particular poison is responsible. Such chemicals as mercuric chloride, lead arsenate, and thallium, used occasionally on the farm, should be stored out of reach of turkeys. While chemical dusts are being applied in sprays or dusts are being applied in orchards where turkeys are ranging, the birds should be removed. After the orchard has been sprayed or dusted, there is still some danger from the residue on the cover crop; if other range is available, the birds should be kept out of the orchard for several additional days or until a rain has reduced the residue remaining on the forage.

A source of poisoning for turkeys, as well as for other fowls, is seed corn or other grains treated for control of fungi. One of these fungicides used in treating seed is Arasan, the active ingredient of which is tetramethylthiuram disulfide (TMTD). Seed corn treated with Arasan contains about 630 p.p.m. of TMTD. It

is hazardous to feed such percentage of the ration even as a small percentage of the ration according to Waibel et al. (1957). Poultry fed Arasan-treated corn developed enlarged hocks, slipped tendons, and an unsteady gait. When rations containing Arasan-treated corn were fed to laying hens, the results were disastrous, simulating acute infectious bronchitis.

Apparently the common weed killers have little or no effect on turkeys if used on vegetation in the usual recommended amounts (Roberts and Rogers, 1957).

REFERENCES

Delaplane, G. F.: 1934. Some of the tissue changes in poultry resulting from the ingestion of sodium bicarbonate. Vet. Alumni Quart. (Ohio State Univ.) 21:149.

DeLay, P. D.: 1940. Grasshopper-poison bait and turkey poult mortality. Jour. Am. Vet. Med. Assn. 97:149.

Hinshaw, W. R., and Lloyd, W. E.: 1931. Studies on the use of copper sulphate for turkeys. Poultry Sci. 10 392.

Hoffman, H. A.: 1912. Unpublished data, used by permission

McNeil, E., and Denny, L.: 1939. Unpublished data, used by permission.

——, and Hinshaw, W. R.: 1945. Effect of mercuric chloride on turkeys and on *Hexamita meleagridis*. Poultry Sci. 24:516.

Roberts, R. E., and Rogers, B. J.: 1957 The effect of 2,4,5-T brush spray on turkeys. Poultry Sci 36:703.

Waibel, P. E., Johnson, E. L., Pomeroy, B. S., and Howard, L. B.: 1957. Toxicity of dimethyl-thiuram disulfide for chicks, poults and goslings. Poultry Sci. 36 607.

Whitehead, F. E.: 1934. The effect of arsenic, as used in poisoning grasshoppers, upon birds. Okla. Agr. Expt. Sta , Bul. 218.

Witter, J. F.: 1956. A preliminary report on the injurious effect of sodium bicarbonate in chicks. Poultry Sci. 15:256.

NEMATODE, CESTODE, AND TREMATODE INFESTATIONS

Since most of the parasitic worms affecting turkeys are also common to chickens, the reader is referred to Chapters 34, 35, and 36 for detailed descriptions of these parasites. Control and treatment are also discussed in these chapters on pages, 995, 1029, and 1052.

Capillaria. Of the several species of the genus Capillaria that infest domestic birds, at least three have been reported in turkeys Two of these, *C. annulata* and *C. contorta*, infest the upper digestive tract, while *two*, *C. caudinflata* and *C. obsignata*, are found in the intestines. Cram (1926) reported *C. annulata* in turkeys in 1926 and later (Cram, 1936) published a comprehensive review on this and other species, giving the principal morphological characteristics of each. Emmel (1939) has described symptoms and necropsy findings observed in the three outbreaks due to *C. contorta*. He calls attention to the penguinlike attitude of infested turkeys (Fig. 41.59). Figure 41.60 shows the gross lesions observed in one of Emmel's specimens. He reported that 5 per cent commercial flowers of sulfur fed in the regular mash caused marked improvement of infested birds in 4 days and prevented new cases from appearing in the flock. The injurious effect of prolonged administration of sulfur is discussed in the sections dealing with coccidiosis and nutrition. *C. obsignata*, formerly known as *P. columbae* (Craybill, 1924; Wehr, 1939b) may cause heavy losses in young turkeys. *C. caudinflata* requires one of several species of earthworms as an intermediate host (Morehouse, 1943). Its true significance as an economic factor in turkey raising is not known. Earthworms are also transmitters of *C. annulata* (Wehr, 1936).

Cecal worms. Cecal worms (*Heterakis gallinae*) are of importance because they act as carriers of *Histomonas meleagridis*, the causative organism of histomoniasis. Recommendations for their prevention will be found in the section on histomoniasis.

Gapeworms. Gapeworms (*Syngamus tra-*
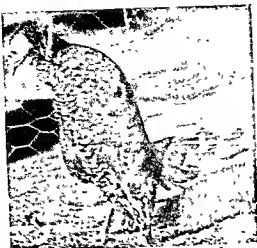
FIG. 41.59—Typical penguinlike posture of a turkey in advanced stage of *C. contorta* infestation. (Emmel, Jour. A.V.M.A.)

chea) cause some mortality in young turkeys, and as shown by Wehr (1939b), survivors may carry the parasite for as long as 224 days. Such survivors are important means of transmission to susceptible chickens and poults.

Ascarids. The intestinal roundworm, *Ascaridia galli*, is not an important parasite of turkeys, and under good husbandry practices the turkey grower need not fear losses from it. Evidence that the turkey is more resistant to the *Ascaridia galli* than is the chicken has been reported by Ackert and Eisenbrandt (1933). Another species, *A. dissimilis*, according to Wehr (Chapter 34), has been reported from both domestic

and wild turkeys in the United States and Cuba. This species is similar in appearance to *A. galli* but is somewhat smaller. Remedies for control of these parasites are not recommended unless definite evidence of a severe flock infestation is found.

Tapeworms may present an economic problem to the turkey grower. Most of the cestodes that infest chickens are also parasitic for turkeys. Prevention, control, and treatment are similar to those outlined for chickens.

Combination anthelmintics designed for removal of both roundworms and tapeworms are not recommended for turkeys. Under no condition should treatment for either type be instituted unless the parasites are known to be causing losses in the flock. Emphasis should be placed on prevention, not on treatment.

Flukes. Riley and Kernkamp (1921), Riley (1931), and Marotel (1926) reported a monostome fluke, *Collyriclum faba*, which encysts in the skin of turkeys and other birds. These usually are found in the abdominal region, and especially in the perianal region, with occasional cysts on other parts of the body (Fig. 41.61) This fluke has been reported in many species of birds by other investigators. Although the complete life history has not been determined, Riley (1931) believes that snails probably act as the first intermediate host and nymphs of dragonflies as the second intermediate
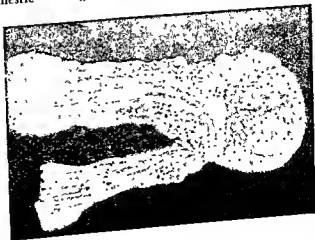
FIG. 41.60 — Crop and esophagus of a turkey suffering from *C. contorta* infestation. (Emmel, Jour. A.V.M.A.)

FIG. 41.61 — A 6-week-old turkey poult showing perianal and abdominal groups of cysts of a fluke, Collyrlclum faba. (Riley and Kernkamp, Jour. A.V.M.A.)

host. English sparrows appear to be important disseminators of the parasites.

Annereaux (1940) reported the occurrence of typhlitis in poults caused by a fluke, Echinoparyphium recurvatum (von Linstow). The 10-week-old poults involved in this outbreak were being ranged along a creek where two types of snails and many tadpoles were present. The lesions found in the ceca of the affected poults were characteristic of those seen in the ceca of poults suffering from blackhead, but no liver lesions were noted. Foggie (1937) has reported an outbreak of parasitic necrosis of intestines of turkey poults in Ireland caused by a fluke, Plagiorchis laricola (Skrjabin), normally a parasite of terns and gulls

According to Macy (1939), the most important species of trematode parasite for North American poultry is Prosthogonimus macrorchis Although not found in natural outbreaks, Macy was able to infect turkeys with this parasite; typical lesions were observed in the oviducts of the parasitized birds. Few external symptoms of disease were noted, but the turkeys ceased laying 4 days after being fed the parasites. This trematode is transmitted by dragonfly nymphs (Lakela, 1932).

Other trematodes reported from turkeys, according to Wehr (Chapter 36), include the following:

Cyclocoelum mutable–Respiratory tract (Europe, Asia, and South America)

Strigea falcoins meleagris–Viscera (Texas)

Postharmostomum gallinum–Ceca (Europe, Asia, Africa, Hawaii, and Puerto Rico)

Notocotylus attenuatus–Ceca (Europe and Asia)

No satisfactory treatment has been reported for trematodes in turkeys. Prevention of infestation consists in avoiding access to marshy pastures, lake shores, or infested streams.

REFERENCES

Ackert, J. E., and Eisenbrandt, L. L.: 1935. On the comparative resistance of Bronze turkeys and White Leghorn chickens to the nematode Ascaridia lineata (Schneider). Jour. Parasit. 20.129.

Annereaux, R. F.: 1940 A note on Echinoparyphium recurvatum (von Linstow) parasitic in California turkeys. Jour. Am. Vet. Med. Assn. 96.62.

Cram, E. B.: 1926. A parasitic disease of the esophagus of turkeys. No. Am. Vet. 7:46.

———: 1936 Species of Capillaria parasitic in the upper digestive tract of birds. USDA Tech. Bul. 516

Emmel, M. W.: 1939 Observations on Capillaria contorta in turkeys. Jour. Am. Vet. Med. Assn. 94:612.

Foggie, A.: 1937. An outbreak of parasitic necrosis in turkeys caused by Plagiorchis laricola (Skrjabin). Jour. Helminth. 15.35.

Graybill, H. W.: 1924. Capillaria columbae (Rud.) from the chicken and turkey. Jour. Parasit. 10 205

Lakela, O.: 1932. Chickens definite hosts to species of Prosthogonimus. Poultry Sci. 11:181.

Macy, R. W.: 1939 Disease in turkeys due to Prosthogonimus macrorchis. Jour. Am. Vet. Med. Assn. 94:537.

Marotel, G.: 1926 Une nouvelle maladie parasitaire: La monostomidee cutanée du dindon. Rev. vét. 78 (12):725.

Morehouse, N. F.: 1944. Life cycle of *Capillaria caudinflata* a nematode parasite of the common fowl. Iowa St. Coll. Jour. Sci. 18:217.

Riley, W.A.: 1931. *Collyriclum faba* as a parasite of poultry. Poultry Sci. 10:204.

———, and Kernkamp, H. C. H.: 1924. Flukes of genus *Collyriclum* as parasites of turkeys and chickens. Jour. Am. Vet. Med. Assn. 64 591.

Wehr, E. E.: 1936. Earthworms as transmitters of *Capillaria annulata*, the "cropworm" of chickens. No. Am. Vet. 17:18.

———: 1939a. Studies on the development of the pigeon capillarid, *Capillaria columbae* USDA, Tech. Bul. 679.

———: 1939b. The gapeworm as a menace to poultry production. Proc. Seventh World's Poultry Cong. p. 267.

ECTOPARASITES

Reference is made to Chapter 33 on External Parasites for a detailed discussion, including prevention and control.

Lice. Turkeys may be infested with the common body louse of chickens, *Menacanthus stramineus* and the chicken shaft louse, *Menopon gallinae.* The large turkey louse, *Chelopistes meleagridis,* and the slender louse, *Oxylipeurus polytrapezius* are probably native to the turkey. The large turkey louse is the most common. Rearing turkeys in close confinement and unsanitary quarters favors lice more than does range rearing. It is important that breeding males and females be examined frequently for lice since parasites may be a very important cause of infertility. A common method of introducing lice to an uninfested ranch is the use of infested shipping crates that have been brought on the ranch by buyers. Growers should insist that buyers clean and disinfect all crates and equipment used to transfer stock to killing plants.

Mites. Turkeys are less affected with mites than chickens, due probably to the greater tendency to rear turkeys out of doors. Chickens reared in close proximity to turkeys, and the use of old chicken yards for turkeys constitute the chief sources of infestation.

Ticks. The only tick of economic importance is the fowl tick or "blue bug," *Argas persicus.* Spirochaetosis, a disease transmitted by this tick, has been diagnosed in the United States by Hoffman *et al.* (1946) in turkeys, and by Burroughs (1947) in chickens. For more details, see the section on spirochaetosis in turkeys, page 1308 It is important that all fowl to be purchased be inspected for the presence of these parasites. Birds (chickens and other fowl as well as turkeys) from infested ranches should never be brought onto tick-free ranches.

REFERENCES

Burroughs, A. L.: 1947. Fowl spirochetosis transmitted by *Argas persicus* (Oken), 1818. from Texas. Science 105:577.

Hoffman, H. A., Jackson, T. W., and Rucker, J. C.: 1946. Spirochetosis in turkeys (a preliminary report). Jour. Am. Vet. Med. Assn. 108:329.

Index